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COMPETITION AND COOPERATION*

Presidential Address

HOWARD C. TAYLOR, JR., M.D., NEW YORK, N. Y.

OBSTETRICS and Gynecology, since its inception as a modern medical specialty, and particularly in the last few years, has been constantly expanding its field of interest and activity. This is a characteristic of all vital intellectual and technical disciplines, and results from the appreciation by their practitioners of the needs and opportunities for application of their special ideas and methods.

Yet during the years when our specialty was growing other areas of medicine were developing to a like extent. It was inevitable then that eventually there would be collision and overlapping of one medical specialty with another and that the nature of Obstetrics and Gynecology would be shaped not solely by its internal forces of expansion but also by the pressures exerted by other medical disciplines about its periphery.

The problem of how to react to the threatened encroachments of other medical disciplines, whether to give ground or press forward, has seemed to me one of the most important that face our specialty. For that reason I have chosen this as the subject of my address, which has been entitled "Competition and Cooperation."

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This situation is of course in no way limited to the field of medicine, for the history of knowledge is as filled with warfare as is any history of nations. One has only to recall the raids of philosophy and religion upon each other; the attempted seizure by sociology of parts of history, psychology, and economics, and the countering efforts of those disciplines to win back their traditional material; or the present no man's land between chemistry and physics where representatives from both sciences are busy staking out competitive claims. Similarly Obstetrics and Gynecology is constantly faced by the claims of other branches of medicine, by the assertion of universal technical competence of the general surgeon, by the occasional intellectual patronage of the internist, by the subtle suggestions of the psychiatrist that *he* understands the psychology of the pregnant woman, and by the pediatrician's persistent efforts to pursue his charge into the prenatal period.

Before analyzing this situation in greater detail, certain reservations must, however, be made. This address is necessarily written from the obstetrician and gynecologist's point of view and by others it may properly be regarded as prejudiced. Furthermore, the competition for areas of work which is referred to is not by any means detrimental to the progress of medicine. Competition should be a stimulus while it lasts and it should end either in the triumph of the arrangement which is better for medicine as a whole or in the adoption of a mutually advantageous system of cooperation.

The area of Obstetrics and Gynecology is, I think, especially vulnerable to encroachment by other medical specialties, and this for two reasons. First, there is no easy method of defining its scope. Second, the field is very large, requiring a wide range of knowledge, both of facts and of techniques, and hence subject to the forces of subdivision.

The means by which the scope of the various divisions of medicine are defined differ considerably from each other. Such a definition may depend on an anatomic area, on the limitations imposed by some highly specialized technique, or, more rarely, and more precariously, on some general concept. Neurology is an example of the first, since even its name implies concern with a single organ system. Urology's position is made almost unassailable by the technical mysteries of the cystoscope. Pediatrics, an example of the third type, makes the fundamental claim that it is the medical science of infancy and childhood.

Obstetrics and Gynecology, as it has matured, has tended less and less to be definable, on a technical or on an anatomic basis, and has tended instead to encompass all of the medical factors which have a bearing on the reproductive process. Such a concept is not limited by techniques, for our work extends far beyond the use of the scalpel or obstetrical forceps. It is not precisely anatomically confined, since the reproductive organs themselves are simply the starting point of physiologic and psychologic change which involves the whole body and the mind as well. The evolution of Obstetrics and Gynecology away from a specialty anatomically defined toward one based on a broad idea is a great

accomplishment, but the abandonment of tangible means of defining our frontiers has made us particularly susceptible to invasion by other disciplines. Cooperation with them has indeed become imperative.

Besides the lack of a criterion for the sharp definition of our branch of medicine, its very extent and heterogeneity make it a difficult area to keep exclusively to ourselves. The union of Obstetrics with Gynecology is an article of faith with most of us, but it has produced requirements in knowledge and skills that stretch human capabilities to their utmost. Between the extremes of cancer of the cervix and toxemia of pregnancy lies most of medicine.

With these general remarks on the competitive nature of the relationship between medical specialties and on the special vulnerability of Obstetrics and Gynecology, one can turn to a consideration of the areas where the problem seems particularly intense. Problems will of course exist to a different degree in different parts of the country and indeed in different individual institutions.

General Surgery.—The claims of General Surgery upon part of the material now handled by the gynecologist are the most insistent. These concern especially the abdominal aspects of pelvic surgery and particularly the treatment of neoplastic disease, which admittedly tends to occur after the reproductive function has been completed.

The arguments offered by the surgeon are of two sorts and are somewhat contradictory. For the benign tumors, especially those requiring simple total hysterectomy, it is argued that no specialized skills are required and that since in many smaller American communities there will be no competent gynecologist the general surgeon must be trained to do this work. The other contention is that the special problems of cancer of the cervix and ovary require not only highly developed skills but so much of the attention of the operator as to more or less preclude his continued interest in the technically unrelated, and distracting, problems of pregnancy and delivery.

These are arguments that are not easily brushed aside, but from our standpoint are quite answerable. The number of obstetrician-gynecologists, adequately trained, is steadily increasing and will in the predictable future be adequate to deal with the great majority of benign pelvic conditions. Furthermore, for the surgical problems arising in women still in the reproductive years of life, it seems essential that decisions as to operation and the nature of the operation be made by those whose thinking constantly turns back to the functional significance of the organs on which he is working. Believing these things, we must nevertheless recognize that the general or abdominal surgeon will occasionally be called upon to carry out some gynecologic procedure and opportunity for some experience in this work should be afforded by the Gynecologic Service to members of surgical residency staffs.

The problem with regard to radical pelvic surgery, especially hysterectomy for cancer of the cervix, presents different aspects. Here the amount of work available is surely insufficient to provide training, still less to maintain the skill of an entire obstetric and gynecologic attending staff. The solution seems to be to select one or more members of the gynecologic staff and assign most of this work to one man or a small group. It is only in this way that a degree of competence can be maintained that will command respect for the gynecologic surgeon.

If exenteration operations are to be undertaken the problem becomes still more difficult, because, although the lesion is indeed gynecologic, the technical steps in the operation require a knowledge of intestinal and urologic surgery.

It is obviously quite possible in an institution which has a large number of cancer cases for a gynecologist, especially if he has had general surgical training, to learn to do this procedure completely, and indeed the dissection of the lateral pelvic walls with avoidance of fatal hemorrhage, which the gynecologist has learned while doing the Wertheim operation, is the most dangerous part of the procedure. Nevertheless, complications are often of a urologic or intestinal nature and it is essential to have the potentially needed consultants favorably disposed. Except in institutions where a point of view or a wealth of malignancy cases makes these procedures common, a pelvic exenteration had better be a cooperative venture.

Urology.—Another field, one which has had great importance in the development of gynecology, seems to me to be in particular jeopardy. This is female Urology and in particular the surgical care of vesicovaginal fistula and urinary incontinence.

The urologist has here the advantage of a concentration of interest, made possible in a relatively small specialty, and he attacks these problems of bladder function and bladder injury with confidence and enthusiasm. Particularly when a problem calls for transvesical exposure of a fistula the urologist has the advantage of familiarity of approach. It is evident that in some institutions our specialty does just as well, particularly perhaps in those in which gynecology is a major interest, but in other services with a wide diffusion of activities these problems of the bladder may be slighted and so pass gradually into the hands of another specialty.

This is a field so traditional to Gynecology that I think we should try to defend it. Simple cystoscopic diagnosis should be one of the gynecologist's abilities. Urinary incontinence and certainly most vesicovaginal fistulas should remain with us. Larger bladder procedures and ureteral operations may well be referred to the urologist or treated in conjunction with him, except in departments of gynecology where there is special attention given to female urology.

Radiotherapy.—Radium was applied to the uterus for the treatment of cancer of the cervix by gynecologists years before the specialty of radiotherapy was dreamed of. Like the treatment of vesicovaginal fistula, radiation for cancer of the cervix is a subject of great traditional interest to us, but our right to it is in some institutions also threatened.

The claim of the radiotherapist is based of course on the greater part now played by external radiation therapy, and by the increasing emphasis being placed on the precise measurement of the radium dosage. There is a real danger that the gynecologist will become simply a pair of hands to dilate the cervix and to insert the radium in the manner of the radiotherapist's instruction.

The solution appears again to be found in the development of one or more members of the gynecologic staff, with the interest and the patience to learn the now complicated principles of radiation therapy and to study each case with the necessary detail. Competition can enter here only to a certain extent and cooperation is essential. The gynecologist will always be superior in his ability to make a diagnosis and to carry out even the minor surgical steps required. The radiotherapist is safe in his control of the apparatus for x-ray therapy and his almost always superior knowledge of radiation dosage. This remains, however, a critical area for the gynecologist and it behooves him to be vigilant and keep up with the times.

Internal Medicine.—The internist approaches the field of Gynecology and Obstetrics in what might be, to us, a more dangerous way. He is concerned with the interaction of pregnancy with various forms of acute and chronic illness. He is particularly fascinated by the syndrome of toxemia of pregnancy, with its clues to the mechanism of hypertension, proteinuria, and edema. He

may even regard gynecologic functional disorders as an extension of the general field of endocrinology which he so clearly considers as a part of internal medicine. The threat from this direction is more serious than that from the surgeon, because it concerns intellectual and scientific aspects rather than technical skills. In our days filled with routine clinical problems, we are tempted to turn over these perplexing questions to a medical consultant or investigator. If this trend were carried to an ultimate conclusion the gynecologist and obstetrician would tend to become a craftsman rather than a medical scientist.

In cases of serious medical illness complicating pregnancy there can be no doubt of the need of the medical consultant. The management of the diabetic, the cardiac, the tuberculous patient is too specialized a responsibility for the obstetrician to undertake entirely himself. There are great advantages, however, in a system in which a more or less permanent medical consultant is assigned to the obstetric service. He gains knowledge of the interaction of disease and pregnancy and becomes far more competent in the handling of these combined problems. He will gradually be partly absorbed into the obstetric service and come to be regarded as a member of the team rather than as an outsider.

The question of who shall care for the patient with toxemia of pregnancy, and more particularly who will direct research in the field, has different aspects from that of the incidental medical complications of pregnancy. Here again is an entity so important to Obstetrics that we must be responsible for the clinical care of these patients as well as for their scientific investigation. At the same time we must be mindful of the fact that new principles in vascular and renal physiology are apt first to reach departments of internal medicine and we should be quick to solicit advice and perhaps collaboration on the application of these principles to the toxemia problem.

Endocrinology is another area of departmental overlapping. Patients with disorders of ovarian function may first appear to present problems of adrenal, thyroid, or anterior pituitary disorder, and conversely patients coming first to the gynecology clinics may display signs and symptoms of primary endocrine disorders elsewhere. The fact that infertility is frequently the complaint which brings the patient to the doctor gives considerable assurance that most of these problems will remain with us. Nevertheless, the rapid increase in scientific knowledge of the hormones regulating reproductive function presents a challenge to Obstetrics and Gynecology to keep up with new advances or this work will go elsewhere. Again the solution seems to be the setting up of a small subdivision within our own departments which will be able to concentrate on this field.

Pediatrics.—Toward the pediatrician we must, I think, accept an almost wholly cooperative attitude. There is no doubt, however, that he has been one of the most successful invaders of the traditional field of Obstetrics. There was a time, I am told, when Obstetrics was even more or less combined with Pediatrics in a specialty known as "the Diseases of Women and Children," in which it was apparently understood that, following delivery, both patients, the mother and the child, remained the professional property of the successful accoucheur. Since that time the pediatrician has steadily and successfully cut down the number of days, hours, and minutes during which the newborn infant is allowed to remain the responsibility of the obstetrician.

Parenthetically, I might report that some time ago in our institution a plastic surgeon had to be summoned as an emergency measure to piece together the results of an operation performed on a male infant on the fifth day of life by a member of the Pediatric house staff who wished to be sure that he had had the experience to handle all the problems of the newborn period.

In spite of this, there are great advantages to the early assumption of the responsibility of the newborn by the pediatrician, advantages based on knowledge

of the special physiology of the infant, a field quite distinct from the maternal physiology which must be the chief preoccupation of the obstetrician. It may be hoped, however, that the pediatrician can be stopped at some point and that he will at least share with the obstetrician the supervision of the undelivered fetus in utero before the onset of labor.

Psychiatry.—Psychiatry is another medical discipline which is beginning to show an interest in our field. Its late appearance is indeed rather surprising, when one recalls the prominent place given to sex and its manifestations by the Freudian theory.

The psychiatrist is now at least developing an interest in more scientific correlations such as relationships between the attitudes of the expectant mother during her pregnancy and her subsequent reactions to her child as well as the possible relationship between measurable phenomena manifested during the first 2 weeks by the newborn and its subsequent development. A few psychiatrists also are showing an interest in functional disorders of menstruation, fertility, and even of early pregnancy.

Our specialty is desperately in need of reliable information in this whole area. The threat is not from the true expert, who will set up objective, well-controlled studies, but from the amateur, professional or even lay, who will deduce emotional relationships on the base of a priori assumptions and a handful of cases or who will endeavor to impose upon us unproved systems for the management of pregnancy and labor.

The Basic Medical Sciences.—Finally, the relationship of Obstetrics and Gynecology to the basic sciences should in every way be a happy one, mutually cooperative and mutually stimulating. There are, however, a few points for comment on the form this relationship may take.

The intensity with which the problems of reproduction are investigated and taught by Anatomy, Physiology, and Biochemistry varies greatly from school to school. In some the subject may be nearly lost sight of. With the hours officially designated to Obstetrics and Gynecology usually relegated to the latter half of the 4 year curriculum, the hours available for aspects of reproduction in the basic sciences become critical in stimulating the student's interest in the subject during the early years at the medical school. The teacher of Obstetrics and Gynecology should do everything in his power to increase emphasis on this subject in the basic science departments, for it is here that the student receives his introduction to our subject.

There is also a place for basic scientific work in the laboratories of the clinical department of Obstetrics and Gynecology itself. The point of view provided is somewhat different from that in the basic science departments, for the human problems are immediately at hand and daily contact with clinicians somewhat orients the research that will be undertaken.

The position of the basic science worker in a clinical department is often a delicate one. In the first place he may feel himself an outsider, professionally and perhaps socially. He may be concerned that his advancement academically in his own discipline is being delayed by identification with a clinical subject. Finally, he may resent his lack of independence in research if his work is completely under the direction of a clinician.

This situation is also soluble, but only by generosity and tact on the part of the clinical obstetrician and gynecologist. In the first place the scientist member of the clinical department must be helped to feel that an academic department of Obstetrics and Gynecology is not simply a school for imparting the techniques of Obstetrics and Gynecology, but is concerned with the study of many aspects of

a vast subject and that he is a full member of the team. To maintain his contact with his own scientific discipline, the device of dual appointments, one in the appropriate basic science department and one in the clinical department, has proved very useful in our institution. Finally, although a laboratory worker may often be brought initially to a clinical department to aid in the solution of some problem already envisaged, there is no way to keep a talented scientist in a clinical department unless he is given a high degree of independence to work on subjects, necessarily within the general field, but beyond that largely of his own choosing.

Comment

Having completed this list of some of the areas in which we are not working alone, areas of competition and cooperation, we can perhaps begin to draw certain conclusions.

First of all, I think, this examination of the periphery of Obstetrics and Gynecology does give us a view of what we are. In the center of the combined specialty is the basic responsibility for the care of the pregnant woman and her delivery, and for the surgery of the female reproductive tract. This is the work we must carry on, which we must teach, and whose techniques we must endeavor to perfect.

But outside of this there is a tremendous new area, made up of new fields of interest, which we must compete for, or share with other disciplines. Much of the work in these new areas is scientific investigation, but in medicine research soon has its effect on practice. Among the areas which are not obviously wholly ours have been noted some phases of radical pelvic surgery, the medical complications of pregnancy, radiotherapy, the newborn, and the infinite amount of basic scientific research that is needed to further the understanding of reproductive physiology. This is only a partial list and one can think of many others, such as obstetrical anesthesia, the problems of fetal physiology and development, a practical genetics, and the many social and psychological problems pertaining to our specialty.

The final question then arises: How can a medical specialty with claims to such dimensions be held together? It is not an easy undertaking, but certainly no more difficult than is being accomplished in the fields of general medicine and general surgery.

A number of ways have already been indicated as to how we may maintain our interests over this large area and deliver a service of such excellence that we can compete successfully.

1. Within our departments there must be some subspecialties, such perhaps as endocrinology, fertility, and cancer therapy. Such subdivisions may be only vaguely demarcated, representing simply the special interests of several members of the clinical obstetrical and gynecological staffs.

2. Next there must be consultants from other disciplines, internal medicine, psychiatry, or anesthesia, attached to the obstetric and gynecologic staffs on a moderately permanent basis so that they come to identify themselves with the specialty and its problems.

3. In some subjects such as the fetus and the newborn, there can be no solution except a frank sharing, but we should be on our guard lest we relax too much and turn over the field completely.

4. Finally, we must attain a close relationship with the fundamental science aspects of our subject, partly by encouraging contacts with the basic science departments of our schools, partly by introducing laboratory research workers into the clinical departments and assuring them proper status to carry on original and independent work.

These are the organizational techniques by which we may hold together our expanded specialty, and oppose the centrifugal forces inherent in the tendency to set up new and independent specialties and the competitive efforts of our colleagues in other branches of medicine. But besides these, and perhaps more important, we have the basic concept of what our specialty represents, namely, the Medical Aspects of the Human Reproductive Process. If we can make good our claims to this idea and assure ourselves that within the framework of Medicine as a whole Obstetrics and Gynecology retains the leadership in its development, we shall not need to be too concerned if in some areas of the general field others also make their contributions.

HORMONES IN HUMAN REPRODUCTION*†‡

Part II. Further Investigation of Steroid Metabolism in Human Pregnancy

M. Edward Davis, M.D., and
E. Jürgen Plotz, M.D., Chicago, Ill.

(From the Department of Obstetrics and Gynecology of the University of Chicago and the Chicago Lying-in Hospital)

IN OUR first report before this society in 1956[6] we presented the results of metabolic studies of progesterone in human pregnancy with the use of the new tracer techniques. We came to the conclusion that the pathway acetate-cholesterol-progesterone-pregnanolone-pregnanediol represents one of the principal physiological routes of synthesis and metabolism of the hormone. We stated that cholesterol of twofold origin is available in the corpus luteum and the placenta for the synthesis of progesterone: (1) cholesterol synthesized within these structures, and (2) plasma cholesterol, which in turn is derived from dietary cholesterol and cholesterol synthesized in the liver. We calculated that about two-thirds of the progesterone synthesized in the corpus luteum and the placenta is derived from plasma cholesterol, and one-third from cholesterol synthesized within these glands. The elimination of progesterone metabolites in the feces as a second important excretory pathway was demonstrated. Furthermore, our results indicated that the hormone disappears swiftly from the circulation, an observation which could not be explained solely by the rapid excretion of its metabolites in feces and urine.

Our report today concerns itself with further investigations concerning the metabolism of progesterone and estrogens in human pregnancy.

Methods

Fourteen pregnant patients were studied. Cancer of the cervix, cardiac failure, multiple sclerosis, cancer of the breast, and other serious maternal complications made therapeutic interruptions necessary in these patients. The studies were carried out during the ninth to the seventeenth week of gestation. Six nonpregnant patients who were admitted for elective pelvic surgery were

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included in these experiments. The amounts of radioactive material administered were approved by the University of Chicago Clinics' General Authorization for Human Use and the Clinics' Radioisotope Committee.

Cholesterol, progesterone, and testosterone, all labeled at carbon atom 4 with carbon[14], were used in these studies. One patient received progesterone labeled at carbon atom 21. All radioactive compounds were purified by chromatographic techniques and repeated recrystallization. Samples of tissues, feces, urine, and blood plasma were oxidized to carbon dioxide in a vacuum combustion line and counted in an ionization chamber, with a vibrating reed electrometer according to the method of Brownell and Lockhart.[3] Unconjugated and conjugated steroids were obtained from the plasma by the method of Bradlow and Gallagher.[2] Samples of expired air were collected in polyethylene bags for periods of 5 to 10 minutes. These samples were transferred to a gas collection apparatus and measured. The carbon dioxide was trapped in aqueous sodium hydroxide. The addition of acid liberated the carbon dioxide from an aliquot of the sodium hydroxide which was measured and transferred to an ionization chamber.

The methods used for the isolation, identification, purification, and radioassay of estrone excreted in the urine of 3 patients are described in detail elsewhere.[8, 22]

Excretory Pathways of Progesterone Metabolites

The early experiments of Venning and Browne[21] demonstrated that the kidneys are important excretory organs for progesterone metabolites (pregnanediol and pregnanolone). Only relatively small amounts of administered large doses of progesterone could be accounted for in the urine of their patients, however. Following the administration of tracer doses of C^{14}-4-progesterone, we were able to recover 25 and 53.6 per cent, respectively, in the urine of our first 2 pregnant patients studied.[5] Most of the radioactivity appeared in the urine during the 24 to 48 hour period following the intramuscular administration of the tagged hormone.

We collected the feces of one of these pregnant patients (C. C.) for 10 days and recovered a total of 28.54 per cent of the administered radioactivity from this material. This observation established the elimination of progesterone metabolites in the feces as another important pathway of excretion in pregnant women. This finding was supported by Wiest and associates,[24] who reported the excretion of 42 per cent of the administered radioactivity in the bile of a nonpregnant woman with T-tube drainage of the gallbladder.

The following experiment[5] was designed to determine whether substantial amounts of radioactivity might be excreted by way of the skin. The fatty film covering the skin surface of the trunk of one of these patients (C. C.) was removed at 6, 18, and 24 hour intervals. There was some radioactivity present in this material (Table I). The total amount present, however, was negligible when compared with that excreted in the feces and the urine (Table II).

TABLE I. RADIOACTIVITY RECOVERED FROM THE FATTY SURFACE FILM OF THE SKIN FOLLOWING THE INTRAMUSCULAR INJECTION OF C^{14}-4-PROGESTERONE

TIME OF COLLECTION (HOURS AFTER THE INJECTION)	EXTRACT FROM MATERIAL OBTAINED BY WIPING THE SKIN SURFACE		EXTRACT FROM MATERIAL OBTAINED FROM GOWN WORN BY PATIENT	
	AMOUNT (MG.)	RADIOACTIVITY DPM*	AMOUNT (MG.)	RADIOACTIVITY DPM*
6	79.6	311	352.3	876
24	69.4	54	420.6	310
48	74.2	75	565.5	106

*Disintegrations per minute.

TABLE II. URINARY EXCRETION OF RADIOACTIVITY IN PREGNANT PATIENTS FOLLOWING THE ADMINISTRATION OF C^{14}-4-PROGESTERONE

PATIENT	WEEKS PREGNANT	DOSE OF RADIOACTIVITY (DPM × 10^6)	TOTAL AMOUNT RECOVERED IN URINE (DPM × 10^6)	PERCENTAGE OF ADMINISTERED DOSE
M. M.	9	5.46	3.05	55.9
N. C.	9	22.5	7.72	34.3
B. M.	10	83.7	27.47	32.8
N. O.	11	5.42	3.34	61.7
C. C.	11	62.4	15.60	25.0
D. V.	11	22.6	8.36	37.0
R. G.	11	21.2	5.47	25.8
T. G.	14	5.46	0.84	15.3
T. O.	17	26.64	14.28	53.6
V. A.	17	5.57	2.52	45.4

Furthermore, we were unable to recover any radioactivity in the carbon dioxide of the expired air of this patient who received progesterone labeled at carbon atom 4, indicating that ring A of the steroid is not split into such small fragments as carbon dioxide and water (Fig. 1). However, the possibility had to be considered that the side-chain of the steroid molecule is removed and oxidized during the metabolism of the hormone. Therefore, we administered a relatively large dose (28.3 μc) of progesterone which was tagged at carbon 21 on the side chain (Fig. 1) to another patient during the tenth week of pregnancy. Substantial amounts of radioactivity were recovered in samples of

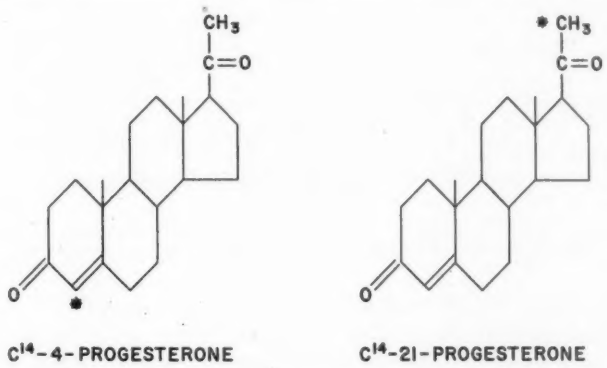

Fig. 1.—Progesterone molecules indicating sites of radioactive labeling.

expired air as shown in Fig. 2. The concentration of radioactivity in the expired carbon dioxide declined rapidly during the first 14 hours and no appreciable amounts could be recovered 31 hours after the administration of the tagged hormone. A rough calculation from these data[14] revealed that approximately 18 to 19 per cent of the administered activity was excreted by way of the lungs within 31 hours after the injection.

This experiment finally established the respiratory tract as the third important pathway of excretion of progesterone metabolites in pregnant women. We determined the total excretion of radioactivity in urine and feces of this patient and found 22.62 and 29.74 per cent, respectively. Thus, we can account for a total of 70.92 per cent of the administered radioactivity in urine, feces, and expired air. At this time we have not accounted for somewhat less than 30 per cent of the injected progesterone. It is highly probable that the unaccounted for progesterone and/or its metabolites are stored in the fat compartments for relatively long periods of time.

The fact that carbon dioxide derived from the side chain of the progesterone molecule is excreted in the expired air indicates the removal of carbon atoms 20-21, resulting in the formation of steroids containing 19 carbon atoms, possibly of the type of Δ^4-androstenedione and its metabolites, androsterone and etiocholanolone (Fig. 3). LeRoy[23] found tagged etiocholanolone in the urine of a patient with adrenal cortical carcinoma who received C^{14}-4-progesterone. The possible interconversion of progesterone into other hormones will be discussed later in this paper.

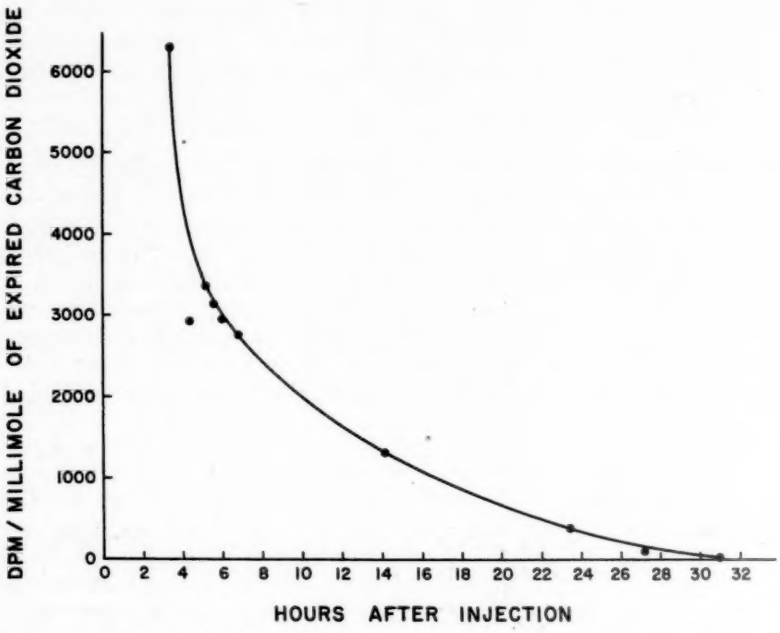

Fig. 2.—Concentration of radioactivity in samples of expired carbon dioxide following intravenous administration of 28.3 μc of C^{14}-21-progesterone.

In 1940, Venning and Browne[21] reported an interesting observation. Following the administration of relatively large doses of progesterone to patients during the luteal phase of the menstrual cycle and during early pregnancy, the recovery of excess urinary pregnanediol was greater than in patients during the proliferative phase, as well as in women with amenorrhea or following bilateral removal of the ovaries. This finding was confirmed by others[18] in spite of the use of different techniques for pregnanediol determination. Thus, in the absence of luteal function, the pregnanediol excretion accounted for less than 15 per cent of the administered dose of the injected hormone, whereas 20 to 40 per cent was excreted as urinary pregnanediol during the luteal phase and in early pregnancy. Furthermore, Guterman[9] demonstrated that the percentage of progesterone converted to pregnanediol was higher in viable than in nonviable pregnancies.

The results of these studies suggested that the rate of conversion of progesterone to its urinary metabolite may have important physiological and clinical implications. Since our first report we have studied the percentage recovery of urinary radioactivity following the intramuscular injection of relatively small amounts of tagged progesterone during the first and second trimesters of pregnancy as well as in nonpregnant women during various states of ovarian function.

The percentages of the injected radioactivity in the urine of 10 patients who were 9 to 17 weeks pregnant are shown in Table II. The amounts excreted varied widely, ranging from 15.3 to 61.7 (average 39.6). There was no relationship between the amounts excreted and the stage of pregnancy or the clinical conditions which made the interruption of pregnancy necessary. A patient with a missed abortion, the fetus being dead for almost 2 months, excreted 50 per cent of the administered radioactivity within 8 days after the injection of C^{14}-4-progesterone (Fig. 4).

The urinary excretion of radioactivity following the administration of tagged progesterone in 5 nonpregnant patients is shown in Table III. The values ranged from 14.9 to 44.6 per cent, with an average of 34.1 per cent. There was no relationship between the amounts excreted and the state of ovarian function.

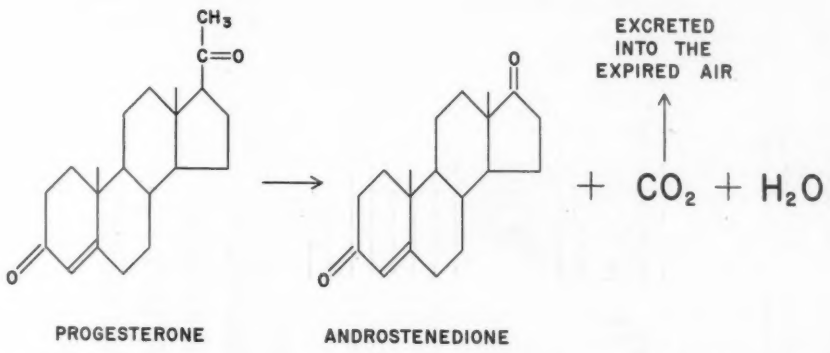

Fig. 3.—One possible mechanism by which carbon dioxide is released by progesterone.

TABLE III. URINARY EXCRETION OF RADIOACTIVITY IN NONPREGNANT PATIENTS FOLLOWING THE ADMINISTRATION OF C^{14}-4-PROGESTERONE

PATIENT	ENDOMETRIUM	DOSE OF RADIOACTIVITY (DPM × 10^6)	TOTAL AMOUNT RECOVERED IN URINE (DPM × 10^6)	PERCENTAGE OF ADMINISTERED DOSE
W. M.	Proliferative	21.0	7.69	36.6
G. S.	Proliferative	21.0	8.88	42.3
N. B.	Secretory	21.2	3.16	14.9
E. W.	Atrophic	22.5	7.20	32.0
A. F.	Atrophic	21.1	9.41	44.6

This lack of correlation between the urinary excretion values of progesterone metabolites and the pregnant and the nonpregnant state, or the viability of the fetus, prompted us to investigate the possibility of an individual excretion pattern. It is known that there is an individual tendency to maintain a fairly constant pattern of excretion of androsterone and etiocholanolone following the administration of testosterone, even though there may be wide variations from subject to subject. The following experiment was designed to investigate the possibility of such individual patterns in the metabolism of progesterone. Five patients received the same amounts of C^{14}-4-progesterone before and after therapeutic abortion (Table IV). Three of these patients excreted approximately the same amounts of radioactivity during both periods and in only one individual was there a considerable decrease in the urinary output following the termination of pregnancy. The percentage recovery of radioactivity in the fifth patient declined after the termination of pregnancy to two-thirds of that found during pregnancy.

TABLE IV. URINARY EXCRETION OF RADIOACTIVITY BEFORE AND AFTER THERAPEUTIC ABORTION FOLLOWING THE ADMINISTRATION OF C^{14}-4-PROGESTERONE

PATIENT	WEEKS PREGNANT	PERCENTAGE OF ADMINISTERED DOSE IN URINE		INTERVAL FOLLOWING THERAPEUTIC ABORTION
		BEFORE INTERRUPTION	AFTER INTERRUPTION	
T. G.	14	15.29	15.53	9 days
R. G.	11	25.78	23.24	5 days
D. V.	11	37.04	44.48	8 days
V. A.	17	45.41	10.02	8 days
N. O.	11	61.73	40.90	7 days

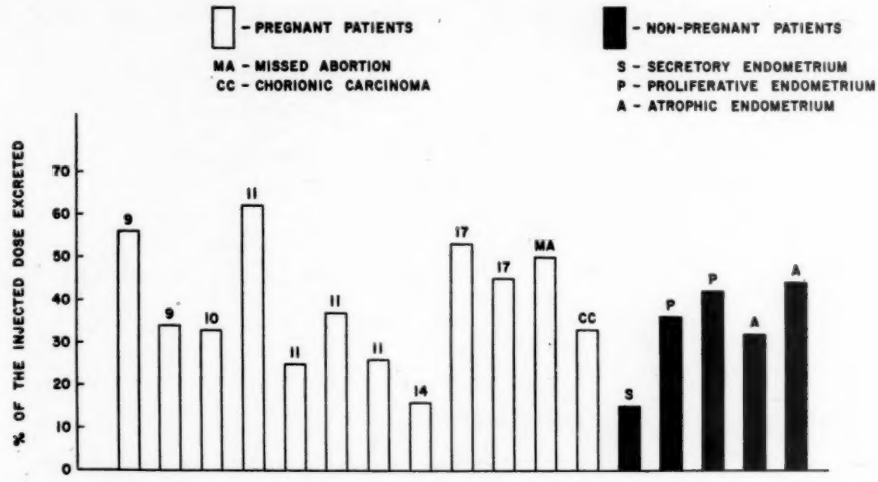

Fig. 4.—Urinary excretion of radioactivity following the administration of C^{14}-4-progesterone to patients with normal and abnormal pregnancies and to nonpregnant patients exhibiting varying endometrial pictures.

Earlier experiments on progesterone metabolism indicated that less than 15 per cent of pregnanediol was recovered in the urine of nonpregnant and 28 per cent in that of pregnant women. Our studies with tagged progesterone however, resulted in a much greater excretion of urinary radioactivity, 34.1 per cent in nonpregnant and 39.6 per cent in pregnant women. This observation indicates that other hitherto unrecognized metabolites are excreted in the urine. One could postulate that in the absence of luteal function the metabolism of progesterone is altered so that the formation of these unknown metabolites predominates, resulting in concomitant decreased conversion to pregnanediol. Thus, the discrepancy between the results of our experiments with the use of tracer progesterone and those where large doses of nonradioactive progesterone were employed would be explained. Quite recently, however, Quilligan and Rothschild[18] reported that they have not seen any relationship between conversion percentage of progesterone to pregnanediol and the absence or presence of luteal function. They also found no relationship between the conversion percentage and the viability of a pregnancy. Further studies of this extremely interesting problem will be carried out in our institution.

We have previously stated that the absorption of progesterone from an oily deposit in the muscle must be a fast and efficient process.[17] This statement was based on the prompt appearance of radioactivity in the urine after the intramuscular administration of C^{14}-4-progesterone to a pregnant woman. The cumulative urinary excretion curve in this patient (Fig. 5) shows a sharp rise

between the sixth and twenty-ninth hours after injection and it becomes almost flat after 96 hours. After the intravenous administration of C^{14}-21-progesterone there is an even sharper rise in the cumulative excretion curve (Fig. 5) and it begins to flatten out about 48 hours after the injection. At the end of the initial 36 hour period 89 per cent of the total urinary radioactivity was excreted by this patient, whereas 71 per cent was eliminated by way of the urine after intramuscular injection during the same period. Although there is no doubt that the urinary excretion rate after intravenous injection is faster than following intramuscular administration, the similarity of both cumulative excretion curves is striking, supporting our previous assertion that the absorption of progesterone from the site of an intramuscular injection is a fast and efficient process.

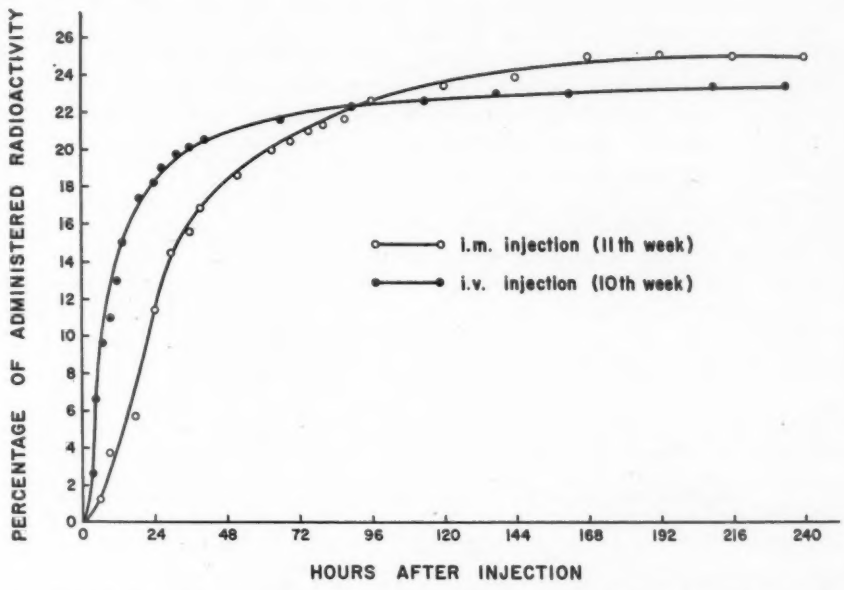

Fig. 5.—Cumulative urinary excretion of radioactivity derived from tagged progesterone after the intravenous and intramuscular injection of C^{14}-4-progesterone.

The Rate of Disappearance of Progesterone and Its Metabolites From the Blood Circulation

In contrast to the large amounts of progesterone metabolites in urine, feces, and expired air, the concentration of the hormone in the blood plasma is very low. Chemical determinations by Zander[26, 27] revealed 0.039 to 0.268 γ per milliliter of plasma, with an average of 0.142 γ per milliliter during the fourth to the ninth month of gestation. There is no progressive increase in the plasma concentration with advancing pregnancy, which is quite in contrast to the increasing amounts of pregnanediol excreted at the same time. When progesterone is administered intravenously, the plasma concentration of the hormone rapidly declines within 2 hours, and no progesterone is detected in the plasma 24 hours later. Pregnanediol, however, continues to be excreted over a greater length of time (2 to 3 days) after intravenous injection. Furthermore, Klein and Ober[13] were not able to increase the plasma concentration of progesterone (as determined by the Hooker-Forbes test) in the blood of pregnant women by intramuscular injections of extremely large doses of progesterone (1,000 to 1,200 mg.), although urinary pregnanediol is excreted in relatively large amounts following such an injection.

All these observations seem to indicate that progesterone secreted into the circulation from its sources of production disappears rapidly from the blood. In our previous communication[6] we reported a peak concentration of radioactivity in the plasma approximately 25 hours after the intramuscular injection of C^{14}-4-progesterone. Assuming that all the counts at peak concentration were obtained from unchanged progesterone, the level of the circulating hormone would have been extremely low. Since there was a close resemblance between the plasma concentration curve and the urinary excretion curve of radioactivity, it seemed reasonable to assume that most of the plasma radioactivity was derived from conjugated metabolites of progesterone.

In a subsequent experiment we administered a dose of 28.3 μc of C^{14}-21-progesterone to a pregnant woman during the tenth week of gestation and determined the concentration of radioactivity present in the conjugated and unconjugated steroid fractions of the plasma. Twenty-five minutes after the intravenous administration of the tagged hormone only 5.75 per cent of the injected dose was present in the total plasma volume as conjugated steroids and only 1.83 per cent as unconjugated steroids (Table V). Negligible amounts of radioactivity remained in the plasma after the extraction of both fractions. The radioactivity concentration curves (Fig. 6) declined rapidly during the first 2 hours and only minimal amounts of radioactivity were present in the unconjugated steroid fraction 37 hours after the intravenous administration of the tagged hormone. It was of great interest to note that within 25 minutes the conjugated steroid level was 3 times as high as that of the unconjugated fraction. This factor increased to 18 and 25 at the 129 and 399 minute time intervals (Table V). Similar findings have been recently reported for nonpregnant women and men by Sandberg and Slaunwhite.[19]

TABLE V. RADIOACTIVITY IN TOTAL PLASMA VOLUME IN THE UNCONJUGATED AND CONJUGATED STEROID FRACTION FOLLOWING THE INTRAVENOUS INJECTION OF C^{14}-21-PROGESTERONE

STEROIDAL FRACTION	PERCENTAGE OF INJECTED RADIOACTIVITY IN TOTAL PLASMA VOLUME*											
	MINUTES					HOURS						
	25	53	129	194	399	12	22	26	37	48	72	96
Unconjugated steroid fraction	1.83	0.79	0.23	0.46	0.10	0.28	–	0.05	0.05	0.10	0.07	0.00
Conjugated steroid fraction	5.75	5.22	4.34	4.19	2.57	1.79	0.80	1.63	0.76	0.60	0.51	0.26
Total	7.58	6.01	4.57	4.65	2.67	2.07	–	1.68	0.81	0.70	0.58	0.26

*Plasma volume = 5 per cent of body weight.

Again, the results of this experiment indicated a rapid disappearance of the free steroid hormone from the blood, an observation which can be partly explained by its extremely fast inactivation to conjugated metabolites. We obtained our first urine specimen from this patient 175 minutes after the intravenous injection of the tagged hormone and recovered 2.78 per cent of the administered dose in this sample. During the same time not more than 3 per cent of the radioactivity was eliminated as carbon dioxide in the expired air. We have no reason to assume that appreciably larger amounts of radioactivity had been excreted by way of the bile during this time interval. Thus, we can account for no more than 10 per cent of the administered dose being eliminated by the kidneys, liver, and lungs within this short time interval after the injection. Nevertheless, only 4.65 per cent of the administered dose was present

in the total plasma volume 194 minutes after the injection. Therefore, it is logical to assume that a major part of the hormone and/or its metabolites diffuses rapidly from the blood plasma into the tissues, from which it is returned more slowly to the circulation and subsequently excreted as metabolites by the kidney and the liver.

The following study was carried out to investigate this possibility. Progesterone labeled at carbon atom 4 was administered to nonpregnant women and pregnant women at varying time intervals before their scheduled operations. The concentration of radioactivity was determined in the tissues removed at operation. The highest concentration of radioactivity was found in the fat specimen obtained from the abdominal wall (Table VI). Assuming an even

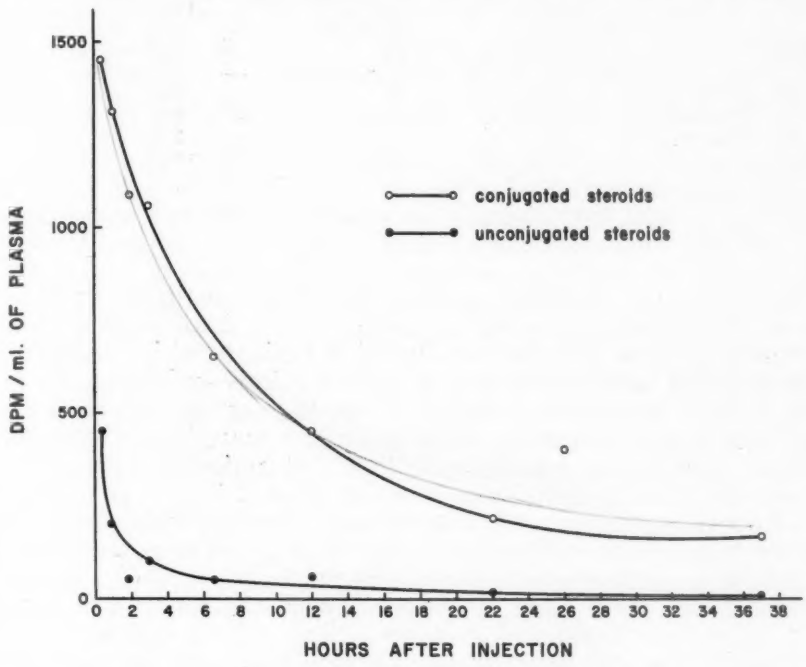

Fig. 6.—Plasma concentration of radioactivity following intravenous administration of 28.3 μc of C^{14}-21-progesterone.

distribution of radioactivity in the fat compartment of the body, about 17.7, 33.7, and 19.6 per cent of the administered dose was present 12, 24, and 48 hours, respectively, after the administration of the tagged hormone in 3 pregnant patients. High concentrations of activity were also found in the fat obtained from nonpregnant women. It is of interest to note that in another case 68 hours after the injection of the tagged hormone about 11 per cent of the administered dose was still present in the fat compartment of the patient's body.

These findings support the thesis that progesterone and/or its metabolites diffuse promptly from the blood plasma into the fat compartment of the body and are apparently retained in this compartment for considerable lengths of time. Our results explain (1) the failure of large doses of progesterone injected intramuscularly to raise the plasma level of progesterone and (2) the delayed excretion of progesterone metabolites after intravenous injection. They further demonstrate that erroneous conclusions may be drawn from values obtained for urinary metabolites of progesterone such as pregnanediol as a measure of

progesterone production. Pregnanediol may still be excreted in the urine in appreciable amounts, although the production of the hormone has already diminished considerably, a supposition which we stressed in our first report when we demonstrated a low rate of synthesis of the hormone from acetate in a patient who was in active labor.

TABLE VI. RADIOACTIVITY IN FATTY TISSUE FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF C^{14}-4-PROGESTERONE

PATIENT	WEEKS PREGNANT	DOSE (μC)	INTERVAL BETWEEN ADMINISTRATION AND REMOVAL OF TISSUE* (HOURS)	CONCENTRATION OF RADIOACTIVITY (DPM/GRAM OF WET TISSUE)	% OF ADMINISTERED RADIOACTIVITY IN TOTAL BODY FAT†
C. G.	11	28.1	48	1759	19.6
T. O.	17	12.0	24	809	33.7
M. P.	18	15.5	12	502	17.7
A. F.	Nonpregnant	9.49	68	249	11.0
N. B.	Nonpregnant	9.56	23	346	20.8
E. W.	Nonpregnant	9.48	23	733	29.4
G. S.	Nonpregnant	10.13	19.5	1072	45.7

*Sample taken from abdominal wall.

†Calculated from body weight of the patient, assuming that 18 per cent of the weight consisted of fat.

In contrast to the high concentration of radioactivity derived from tagged progesterone in fat only moderate amounts of radioactivity were demonstrated in the myometrium and the decidua of 2 patients with advanced pregnancies (seventeen and eighteen weeks), whereas a relatively higher concentration was found in these tissues of a patient 11 weeks pregnant (Table VII). The concentration of activity in the decidua and myometrium of the latter patient, however, was still considerably lower than that in the fat. Similar low concentrations of radioactivity were present in the myometrium of nonpregnant patients. It is of great interest to note that extremely low concentrations of radioactivity could be found in the endometrium of 2 women who were operated upon during the pre- and postovulatory phases, respectively, of their normal menstrual cycles. On the other hand, no radioactivity whatsoever was demonstrable in the atrophic endometrium of 2 other patients. It is well known that endometrium which has not been primed by estrogens remains unresponsive to the typical biological effects of progesterone. Our findings seem to indicate that, in the absence of estrogen activity, progesterone and/or its metabolites do not penetrate into the cells of the mucosal layer of the uterus. Our results furthermore demonstrate that only minimal amounts of progesterone are necessary to exert its biological effect on the target organ, the endometrium.

At the present time, we have no definite knowledge about the actual mechanism by which a steroid hormone exerts its effect on the target organ. It is entirely possible that progesterone originally secreted by an endocrine gland is metabolized to other compounds, which finally produce the so-called progestational effect on the target organ at very low concentrations. Progesterone itself could not be isolated from the myometrium and the decidua of pregnant patients when a very sensitive method of chemical assay was used.[28] On the other hand, Sweat and co-workers[20] demonstrated the in vitro conversion of progesterone into Δ^4-3-ketopregnene-20-α-ol and Δ^4-3-ketopregnene-20-β-ol by human endometrium. Both compounds possess progestational activity in the Hooker-Forbes and the Clauberg tests as shown by Zander and associates.[29] The same authors recently reported the isolation of these new biologically active metabolites of progesterone from human ripe follicles, corpora lutea,

placentas, and fat tissue. In addition, other unknown conversion products of progesterone may have important hormone activities at the molecular level within the cells of the target organs.

TABLE VII. CONCENTRATION OF RADIOACTIVITY IN TISSUE FOLLOWING THE ADMINISTRATION OF C^{14}-4-PROGESTERONE

PATIENT	FAT	ENDOMETRIUM	MYOMETRIUM	MYOMA
	(DPM/GRAM OF WET TISSUE)			
C. C.	1,754	594 (decidua)	809	
J. O.	809	136 (decidua)	90	
M. P.	502	101 (decidua)	196	
G. S.	1,072	85 (proliferative)	300	630
N. B.	346	18 (secretory)	77	218
A. F.	249	0 (atrophic)	163	
E. W.	733	0 (atrophic)	13	113

Studies on the Synthesis of Estrogens in Human Pregnancy*

It was stated in our first report that we did not recover labeled estriol from the urine of 2 pregnant women who received cholesterol labeled with radioactive hydrogen (tritium). Further attempts with tritium cholesterol were likewise unsuccessful. These observations seemed to support the findings of Heard and O'Donnell,[10] who found that estrone was not synthesized from C^{14}-labeled cholesterol in the pregnant mare. Relatively small amounts of tagged cholesterol were used in our initial experiments, however. Therefore, we administered a total of 87.4 μc of C^{14}-4-cholesterol over a period of 6 days, during which time all the urine was collected and processed as a single pool.[22] The crude phenolic fraction, containing the estrogens, revealed 0.012 per cent of the administered activity. By the use of large amounts of carrier, radiochemically pure estrone was recovered. The radioassay data are shown in Table VIII. The specific activity of the final compound remained constant when its derivatives (estrone acetate and benzoate) were formed. The free estrone recovered had been diluted in the isolation process by approximately 75 parts of carrier. The approximate specific activity of the patients' estrone was 0.190 μc per millimole.

TABLE VIII. SPECIFIC ACTIVITY OF C^{14}-ESTRONE (ISOLATED BY CARRIER METHOD) FOLLOWING THE ADMINISTRATION OF C^{14}-4-CHOLESTEROL

COMPOUND	MG. COUNTED	DPM*	FREE ESTRONE DPM/MG.
Estrone acetate (I)	117.4	1,615	15.9
Estrone acetate (II)	96.1	1,335	16.0
Estrone benzoate (I)	84.7	899	14.7
Estrone benzoate (II)	77.0	806	14.5
Estrone benzoate (III)	68.9	761	15.3

*Corrected for quenching effects.

The results of our experiment demonstrated that estrogens can be synthesized from cholesterol in human pregnancy, and lends support to the theory that this sterol is an important precursor of most if not all steroid hormones, since it had been incorporated into such compounds as pregnanediol, androsterone, etiocholanolone, cortisone, cortisol, tetrahydrocortisone, 11-keto-androsterone, and other metabolites of steroid hormones in in vivo and in vitro experiments (Fig. 7).

*Drs. Roy Hertz and Delbert M. Bergenstal of the Endocrine Section, National Institutes of Health, Bethesda, Maryland, cooperated in this study.

Studies of various investigators[1, 11, 15, 25] have led to the conclusion that androgens of the type of testosterone and androstendione may function as precursors of estrogens in the human nonpregnant female. The following study was designed to investigate whether such a conversion is in actual operation during pregnancy. A dose of 35.09 μc of C^{14}-4-testosterone was administered intramuscularly to a patient scheduled for therapeutic abortion during the seventh week of gestation.[8] About 55 per cent of the radioactivity derived from the tagged androgen was recovered in the urine during the first 3 days after injection. In the crude phenolic fraction a total radioactivity of 0.276 μc (0.8 per cent of the administered dose) was recovered. With large amounts of carrier pure estrone was identified. As seen in Table IX, this estrone was radioactive. The specific activity of its acetate remained constant before and after chromatography, as well as after subsequent hydrolysis to the free steroid, indicating the radiochemical purity of the compound.

TABLE IX. SPECIFIC ACTIVITY OF C^{14}-ESTRONE (ISOLATED BY CARRIER METHOD) FOLLOWING THE ADMINISTRATION OF C^{14}-4-TESTOSTERONE

COMPOUND	MG. COUNTED	DPM*	FREE ESTRONE DPM/MG.
Estrone acetate (I)	39.1	1,171	29.9
Estrone acetate (II)	17.8	563	30.6
Estrone	6.7	194	28.9

*Corrected for quenching.

Our data lend support to the conclusion that the metabolic pathway cholesterol-testosterone-estrone is one of the routes of estrogen biosynthesis in human pregnancy. Our data do not permit the determination of the exact yield of estrone from testosterone, but it was certainly less than 0.8 per cent since the combined crude phenolic fraction containing almost all estrogens revealed only 0.8 per cent of the radioactivity administered.

The question arises as to where androgens like testosterone or Δ^4-androstendione are produced and where they are transformed to estrone in the human pregnant organism. To our knowledge there is no evidence that the placenta can synthesize androgenic hormones, but in vitro experiments by Meyer[15] have shown that human placental tissue is capable of converting Δ^4-androstenedione into estrone with a yield of 0.5 to 1.3 per cent of estrone. It is possible, therefore, that androgens used for estrogen synthesis within the placenta are produced by other sources such as the fetal adrenal glands, or eventually, the ovaries and adrenals of the mother. In this connection, it is certainly of interest to note that, at the peak of estrogen excretion during the third trimester of human pregnancy, the principal metabolites of androgens (androsterone and etiocholanolone) are excreted in decreasing amounts.[7] By the eighth month, their excretion is very much reduced, and androsterone may disappear from the urine in some cases. It is conceivable that the decreased urinary excretion of androgen metabolites is due to the utilization of their hormonal precursors in the synthesis of estrogens at this period in pregnancy.

Since it is known from the results of several in vivo and in vitro studies that progesterone can be converted into androgens and, in turn, androgens into estrogens, one could assume that progesterone may serve as a precursor of estrogens. So far we have not been able to demonstrate a progesterone-estrone conversion in normal pregnancies. When 37.81 μc of C^{14}-4-progesterone was injected intramuscularly into a patient with metastatic choriocarcinoma, however, 0.434 μc (1.1 per cent) of the administered dose was found in the crude phenolic fraction. After carrier was added pure estrone was isolated and

radioassayed. The counting data are shown in Table X. The specific activity of this material remained constant when its derivatives were formed, indicating radiochemical purity.

This observation might indicate a specific alteration of the steroid metabolism in patients with choriocarcinoma. It is known from the studies of Kaufmann and Zander[12] that choriocarcinomatous tissue exhibits a high concentration of progesterone as determined by chemical assay. On the other hand, only traces of or no pregnanediol is being excreted into the urine of these patients as shown by Plotz[16] and others.[12]

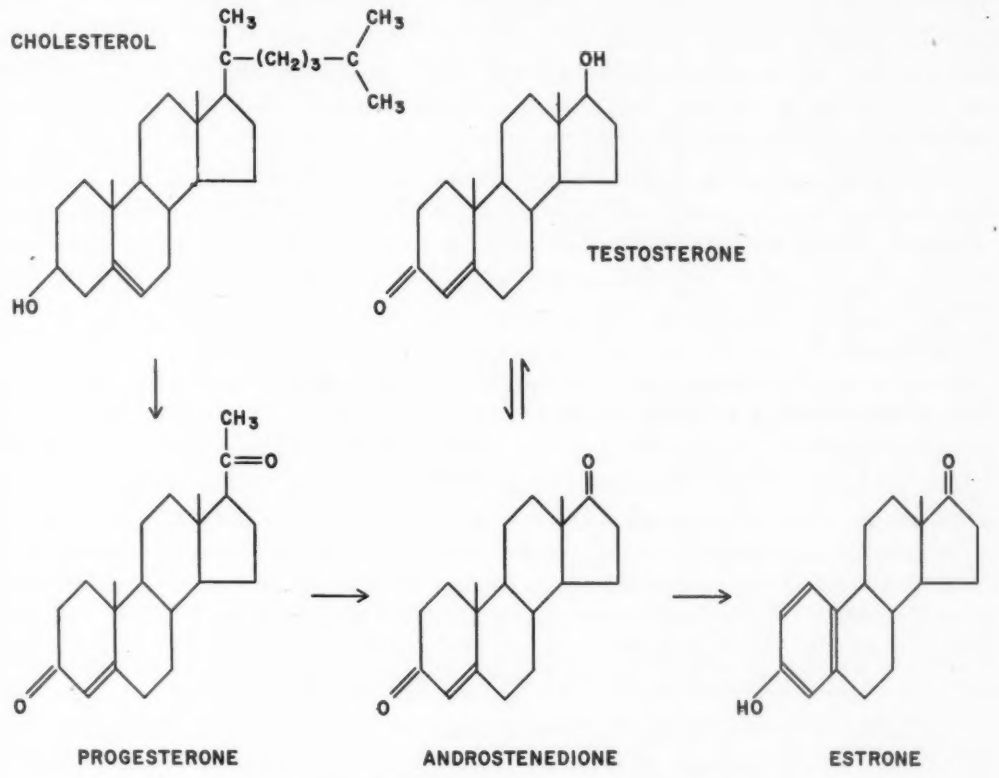

Fig. 7.—Interconversion of hormones.

TABLE X. SPECIFIC ACTIVITY OF C^{14}-ESTRONE (ISOLATED BY CARRIER METHOD) FOLLOWING THE ADMINISTRATION OF C^{14}-4-PROGESTERONE

COMPOUNDS	MG. COUNTED	DPM*	FREE ESTRONE DPM/MG.
Estrone benzoate	260.2	549.9	2.93
Estrone (first crystallization)	180.1	523.8	2.91
Estrone (second crystallization)	175.5	515.5	2.93
Estrone acetate	172.1	499.4	3.33

*Corrected for quenching effects.

Summary

Following the administration of progesterone labeled with carbon[14] at position 21 to a patient with a normal pregnancy about 70 per cent of the administered radioactivity was recovered from the urine, feces, and expired air

(22.62 per cent, 29.74 per cent, and 18.56 per cent, respectively). The excretion of radioactive metabolites by the skin was minimal. At present we have not accounted for the remaining 30 per cent of the administered radioactivity, but it seems likely that part of it is stored in the fat compartments of the body for a considerable length of time. There was no relationship between the urinary recovery of radioactivity and the pregnant and nonpregnant state, nor the viability of the fetus.

The metabolites in urine and feces consist mainly of conjugated steroids, as pregnanediol, pregnanolone, and other unknown compounds. The elimination of tagged carbon dioxide after the administration of C^{14}-21-progesterone indicates an oxidation of the side chain of the progesterone molecule, resulting in the formation of such steroids as androstenedione and related compounds which have androgenic properties.

Determinations of radioactivity in the blood plasma following the intravenous and intramuscular administration of the labeled hormone demonstrated a rapid disappearance of the free steroid from the circulation due to (1) a speedy conjugation with glucuronic acid and (2) rapid diffusion into body tissues, mainly into the fat compartment. In contrast to the high concentration of radioactivity in the fat, only moderate amounts of radioactivity were demonstrated in the myometrium and the decidua of pregnant patients. Extremely low concentrations of radioactivity were found in the endometrium of nonpregnant women during the pre- and postovulatory phases of the menstrual cycle, and no radioactivity was demonstrable in the atrophic endometrium of 2 patients. The absence of estrogenic activity seems to prevent the entrance of the hormone into the cells of the mucosal layer of the uterus. This observation provides direct evidence that estrogens must prepare the endometrial cells in order that progesterone can exhibit its progestational effect. Apparently, only minimal amounts of progesterone and/or its metabolites are necessary to exert its biological effects on the endometrium.

Preliminary studies on the biosynthesis of estrogens in human pregnancy revealed that cholesterol as well as testosterone can serve as a precursor of estrone. In a patient with choriocarcinoma C^{14}-4-progesterone was converted into radioactive estrone.

The data which we have presented here complete another milestone in the very long experimental road toward our understanding of the metabolism and biosynthesis of the steroid hormones in mammalian reproduction. It becomes increasingly apparent that our thorough understanding of their role in the normal reproductive process must precede an intelligent approach to the solution of many problems in obstetrics and gynecology.

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Discussion

DR. C. LEE BUXTON, New Haven, Conn.—Every one of the many aspects of this paper could easily provoke lengthy discussion. Therefore, in a brief time it may be advisable to concentrate more on its more general physiologic aspects.

This discussion of steroid metabolism in the female, as it has been presented here by Drs. Davis and Plotz, does not so much involve pregnancy or even cyclic menstrual changes as it does the fate in the organism of tagged steroids in general and, more specifically, progesterone tagged by two techniques. This fashion of investigating the fate of progesterone and its metabolites presents a wealth of information which was hitherto unavailable.

Whereas by previous clinical measuring techniques only about 10 per cent of progesterone metabolites could be recovered in the urine, this process presents a recovery rate of 25 to 50 per cent and also confirms previous work which indicated that bile was an important pathway of progesterone metabolite excretion.

The wide variation in amount of urinary excretion of the tagged material in both the pregnant and nonpregnant individual, however, is quite surprising. It is my understanding that radioactive studies with other hormones and apparently other techniques show fairly stable values. This must represent considerable patient variability and I wonder if, in spite of the essayists' remarks to the contrary, the systemic condition of the patients, for which therapeutic abortions were performed, for instance, might not be of "excretory significance."

The most intriguing details of the rapid disappearance of tagged material from the plasma after both intravenous and intramuscular injection, and the confirmatory evidence of work done by Zander and others that the body uses fat as a storage space for progesterone and its metabolites, and other steroids also, lead to lots of interesting speculation.

The equally intriguing finding that an important end organ of progestational activity, the endometrium and the myometrium, contains but little of this material seems to indicate that, its purposeful mission having been accomplished, it is then probably

excreted. I would not agree that the evidence presented in this paper demonstrates any significant difference in endometrial capacity for storage depending on whether or not this tissue is or is not estrogen primed.

The authors comment on the fact that we as yet have no idea of the actual mechanism by which a steroid exerts its effect on a target organ, but it seems to me that it is studies such as this which may lead to a revelation of this mystery.

There is much material in this paper which will undoubtedly be discussed extensively by steroid chemists and upon which I certainly am not qualified to comment.

For those of us who are fascinated by the apparently almost identical chemical structure of the different gonadal and adrenal steroids compared to the radical differences in their physiologic activity this paper presents a further stimulating thought. There is expressed here by implication as well as factually the possibility of extensive physiologic and, therefore, undoubtedly occasionally pathologic conversion of one steroid into another. The conversion of progesterone to 17-hydroxyprogesterone, a precursor of androstenedione, indicates the close relationship between progesterone and male sex hormones. And the conversion of radioactive testosterone to C^{14}-estrone may well take place elsewhere than in the placenta and, therefore, in the nonpregnant patient. Recent reports by Sweat, by Zander and Kaufmann, and now this outstanding contribution by Davis and Plotz suggest a steroid interrelationship which may help to explain the cases of hirsutism, masculinization, and amenorrhea in the female in whom we have difficulty finding morphologic pathologic changes.

DR. DAVIS (Closing).—We realize that our investigations have raised many questions and provided few answers to the many problems which we encounter daily in the clinical care of our patients.

The biological sciences and medicine today stand at the same place that the physical sciences occupied prior to the splitting of the atom. Atomic fission ushered in a new physical world with horizons unlimited. The biological sciences are likewise on the threshold of a new world. In the place of the atom our scientists are unlocking the secrets of the cell; its origin, its metabolic function, its response to environmental change, its reproduction. The steroid hormones and their enzymatic controls are undoubtedly fundamental factors in the cellular function of the reproductive organs. To understand the cell and its multiple facets will provide basic information which will help us to understand the organ from which it comes, and ultimately, the human body in health and disease.

ELECTROLYTE BALANCE STUDIES WITH THE ANTIHORMONES* †

ALLAN C. BARNES, M.D., AND JOHN C. BUCKINGHAM, M.D.,
CLEVELAND, OHIO

WITH THE TECHNICAL ASSISTANCE OF JAMES DILLHOEFER

(From the Department of Obstetrics and Gynecology, Western Reserve University School of Medicine)

IN the maintenance of water and electrolyte homeostasis, the steroid hormones exercise the principal control over renal electrolyte management and obligatory water retention. Comparative studies of the premenopausal versus the postmenopausal woman, or of the adrenalectomized versus the intact patient indicate the long-range metabolic effect of the withdrawal of various steroid components from the body economy. Yet similar studies on the immediate effects of declining levels of the steroids are not readily available. From the observations by the Thorns[1] of the sodium-retaining effect in dogs of the ovarian hormones, through Dignam's[2] careful studies of the electrolyte alterations in human subjects resulting from the administration of estradiol, there have been many studies of the acute changes resulting from increasing steroid levels. These have indicated that in general the ovarian hormones have an electrolyte effect which is qualitatively similar to, but quantitatively less than, the effect of the adrenal cortical hormones. As a rule, conclusions as to the immediate effect of the withdrawal of these steroids have postulated that the opposite electrolyte changes take place.

The introduction of antisteroid substances—both the antialdosterones and the antiestrogens—potentially offers the opportunity to study electrolyte changes resulting from the selective suppression of individual endocrines.

Material and Method

The present report is drawn from approximately 50 metabolic balance studies on obstetric and gynecologic patients. Included have been patients in the immediate puerperium—normal and toxemic—experiencing the declining levels of both the placental and the corticosteroids; young women who had been subjected to bilateral oophorectomy; and women receiving both antiestrogen and antialdosterone medications.

*This study was carried out with the aid of grants from the Cleveland Area Heart Society and the Prentiss Foundation.

†Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

The balance studies were carried out in the usual manner with all output (including lochia and breast milk) collected and measured. The electrolyte determinations were made on the flame photometer (for sodium and potassium), by the Van Slyke modification of the Sendroy[3] method (for chloride), and macro-Kjeldahl (for nitrogen). The principle employed for intake measurements involved the "weigh back" technique; that is to say, rather than offering prescribed amounts of the electrolytes involved, each patient was presented with an excess of each element, free selection was permitted, and the unconsumed portion weighed and subtracted from the total. The result of this approach is a much greater variation in the day-to-day intakes (with some resultant influence on the balance picture), but it permits greater palatability and some interpretation of the patient's established eating habits. Cornification counts were obtained after Shorr's stain, and the excretion of sodium-retaining factor measured by assaying in adrenalectomized rats chloroform extracts of the patient's urine.[4]

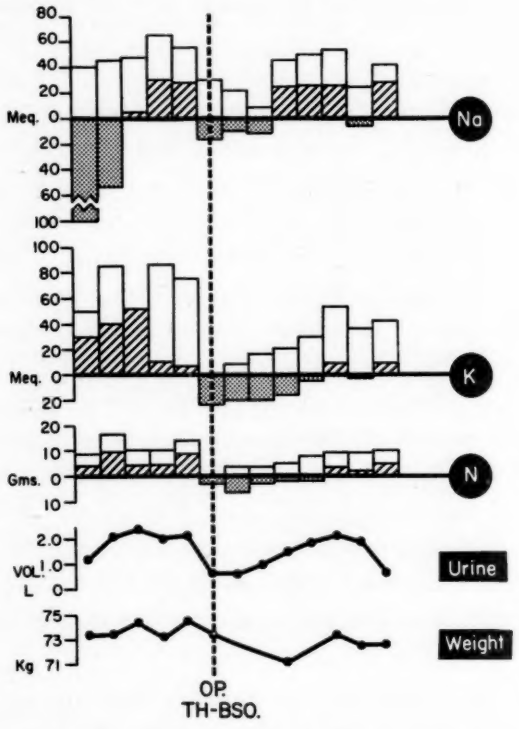

Fig. 1.—Postoperative balance studies of a postmenopausal woman after total hysterectomy and bilateral salpingo-oophorectomy. For each day the intake is charted from the base line up, and the output superimposed, charted from the top of the intake bar downward. When the output exceeds the intake, this excess is shaded beneath the base line; when the output is less than the intake, this difference is cross-hatched above the base line.

Effects of Stress

Surgery.—Control patients undergoing nonpharmacologically induced withdrawal of steroids are not easily evaluated, since the surgical procedure to remove the gland of production may well obscure the effect of the declining levels of steroids. The fact that operative procedures have a reasonably constant impact on the patient's sodium, potassium, and nitrogen levels is well documented; it is the anticipated effect of stress, and can be reduplicated by the administration of ACTH to the normal subject.[5, 6]

The balance study in Fig. 1 can be considered representative of the impact of surgery itself on the patient's electrolytes. The patient was a postmenopausal woman who had a total hysterectomy and bilateral salpingo-oophorectomy. Presumably the actual change in the level of ovarian steroids would be small. The characteristic changes of sodium retention with a negative balance of potassium and nitrogen are typical of the response to surgery.[6]

The patient whose metabolic studies are recorded in Fig. 2 has been subjected to the same surgical procedure as was the patient whose findings are depicted in Fig. 1. The fundamental difference, however, is that the patient represented in Fig. 2 was menstruating regularly at the age of 36 and following operation would be experiencing a declining estrogen level as a result of the loss of functioning ovaries. Despite this declining level of ovarian steroids (which, judging from the onset of symptoms of vasomotor instability, must have been exceedingly gradual) the patient presents fundamentally the same type of picture as indicated in the postmenopausal woman represented in Fig. 1. Eight other women's studies confirm the findings illustrated by these 2 examples.

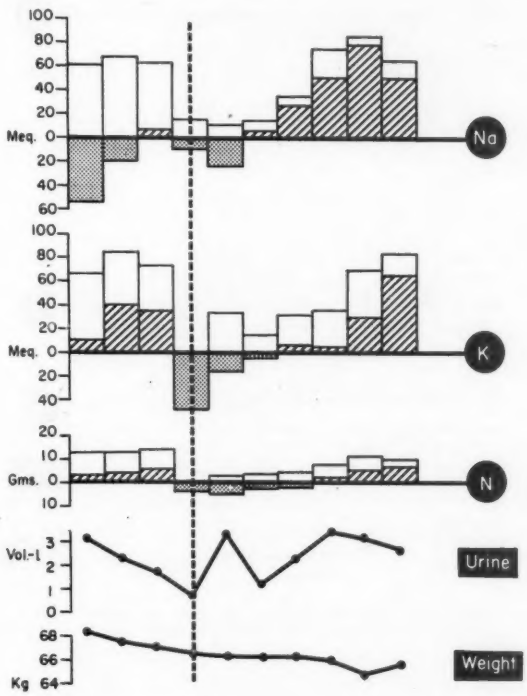

Fig. 2.—Postoperative balance studies following total hysterectomy and bilateral salpingo-oophorectomy in a regularly menstruating woman.

The Puerperium.—The most marked withdrawal of steroids is represented by delivery. While the estrogen and progesterone levels start to diminish before labor, their most abrupt decline occurs during the immediate days after parturition.

Fig. 3 indicates the electrolyte balance studies for the first 6 puerperal days. Immediately after delivery the patient's urinary output is high and she is losing an excess of sodium while retaining both potassium and nitrogen. The pattern for each of these ions is the reverse of that seen in the first few days after gynecologic surgery.

It is not possible to conclude from a comparison of Figs. 1 and 3 that labor and delivery do not represent stress or an alarm reaction. The typical electrolyte pattern of increased corticoid activity in the postoperative woman is masked, however, by the more dramatic decline in the total steroid levels associated with the delivery of the placenta.[7]

Results With Aldosterone Antagonist

During pregnancy there is increased activity of the adrenal cortex with a rising excretion of corticoids. Particularly aldosterone has been found—most consistently in the urine of women with pre-eclampsia and eclampsia—but also in nontoxic pregnant women in the last trimester.[8] In a consideration of the metabolic readjustment of the early puerperium, one of the significant influences contributing to the observed changes is undoubtedly the declining level of this

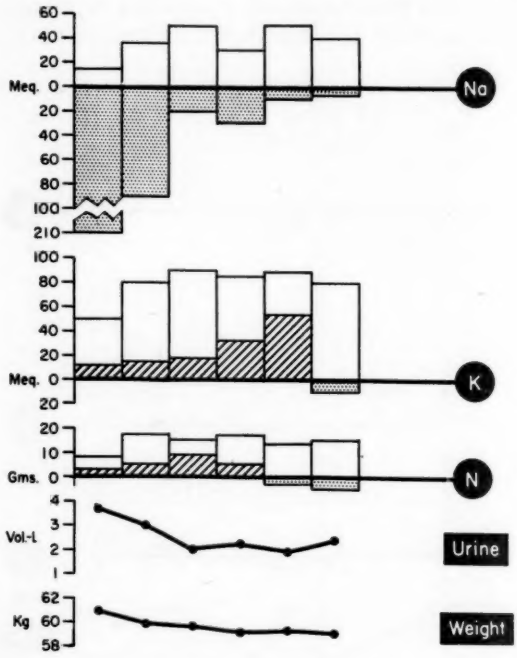

Fig. 3.—Balance pattern for the first few puerperal days. The studies were started 12 hours after delivery, and the patient's oral intake had been minimal the 24 hours before measurements were started.

steroid. The nor analogue of 3-(3-oxo-17B-hydroxy-4-androsten-17γ–yl) propionic acidγ-lactone (Fig. 4) blocks the sodium and potassium effects of aldosterone. Supplied under the designation SC-8109,* this agent has been tested in patients with congestive failure and nephrosis, as well as in normal subjects on sharp salt restriction (to increase their endogenous elaboration of aldosterone).[9, 10] The drug has little effect on the electrolyte excretion pattern in control subjects; in the face of increased levels of aldosterone, however, it induces an increased elimination of sodium with a reversal of the Na/K pattern produced by aldosterone.

To evaluate the effect of a selective blockade of aldosterone in pregnancy, SC-8109 was administered to 2 normal women in the last month of pregnancy,

*G. D. Searle & Co., Chicago, Ill.

and to 4 women with pre-eclampsia of varying degrees of severity. In addition to the water and electrolyte balance studies, daily assays of the sodium-retaining factor in the patients' urine were carried out.

Fig. 5 indicates a characteristic change in one of the control patients. This woman, a 26-year-old primigravida, had had an uneventful pregnancy without either excessive weight gain or hypertension. As is customary with our antepartum instructions, she had been on a limited intake of salt to the extent that she had been requested to use cooking salt in moderation and to add no salt to her food at the table. She was admitted to the metabolic balance ward at 38 weeks of pregnancy.

Despite the nontoxemic clinical course that the patient had followed, it can be seen that after obtaining a positive sodium balance by the third day of study the administration of SC-8109 resulted in a prompt and dramatic reversal of the sodium elimination with a tremendous negative sodium balance during the days that the antialdosterone medication was given. As is true of the postpartum

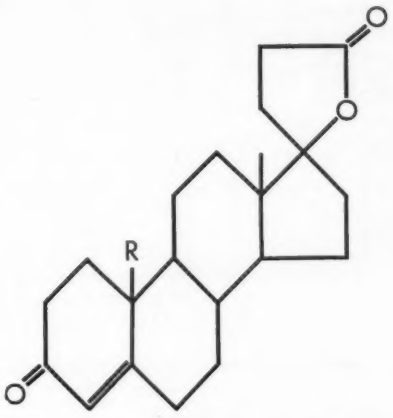

Fig. 4.—Structural formula of steroid with aldosterone-blocking effect. In steroid SC-8109 (tested in present paper), R is H.

patient, the potassium and nitrogen balances tend to shift in the opposite direction from the sodium, and the chloride balance, although inconstant, tends to follow the sodium elimination. This electrolyte response was typical of other nontoxemic controls.

The patient whose studies are indicated in Fig. 6 was likewise in the last month of pregnancy but in contrast was carrying a blood pressure of 150/110 with a 2 plus proteinuria and 1 plus clinical edema at the time she was admitted for a balance study. In this patient, as with others admitted with the clinical diagnosis of pre-eclampsia, the effect of the antialdosterone medication in the dosages used was considerably less impressive. In each instance, considering the day of medication and the following day as representing the maximal period of drug effect, it can be seen that the patient with pre-eclampsia continued to maintain a positive sodium balance and the dramatic natriuresis seen in Fig. 4 did not result from the administration of the antialdosterone. Whatever the mechanism of aldosterone antagonism of SC-8109 it is entirely possible that the contrast between Fig. 5 and Fig. 6 simply represents inadequate dosage. During treatment it was noted that while the patients with pre-eclampsia had some complaint of lethargy, they had fewer side effects in the form of intestinal cramping, anorexia or loose stools than did the normal pregnant controls. Clinically in none of the patients studied did the course of the eclamptogenic toxemia appear to

improve significantly, although the assays of the sodium-retaining factor in the patients' urine indicate a diminished excretion of this factor under the influence of SC-8109 (Fig. 7).

Results With Estrogen Antagonist

The estrogen antagonist selected was 1-(*p*-2-diethyl amino ethoxyphenyl)-1-phenyl-2-*p*-anisylethanol (Fig. 8). It is a nonsteroid substance which effectively blocks some of the effects of estradiol in the laboratory animal. Administered simultaneously with 2 γ of estradiol benzoate to the castrated rat, 5 mg. of MER-25* in 2 equal doses prevents the increase in vaginal cornification count or the increase in weight of the uterus which the unopposed estrogen produces in the

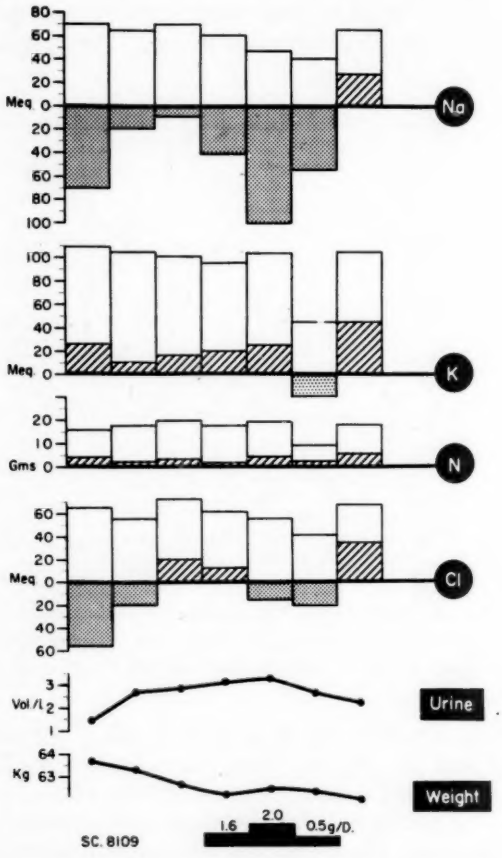

Fig. 5.—Balance studies of a normal primigravida in the last month of pregnancy. The clinical course of the pregnancy had been without evidence of toxemia. The days the anti-aldosterone was administered are indicated below, and are characterized by a marked shift in the Na/K ratio.

controls (Fig. 9). In seeking to determine whether or not this drug would also blockade the electrolyte effects of estrogens, it was administered orally to women in the balance ward in doses of from 1 to 2 Gm. a day. At a dosage level of 2 Gm. a day, some headache and dizziness were experienced by about half the patients after several days of treatment.

*The Wm. S. Merrell Co., Cincinnati, Ohio.

In a series of 3 regularly ovulating and menstruating women, this drug in tolerated doses had no significant effect on the vaginal cornification count. In one 28-year-old regularly menstruating patient, a dose of 2 Gm. a day for 5 days produced an atrophic endometrium; smaller doses (1.0 or 1.5 Gm. daily) for 3

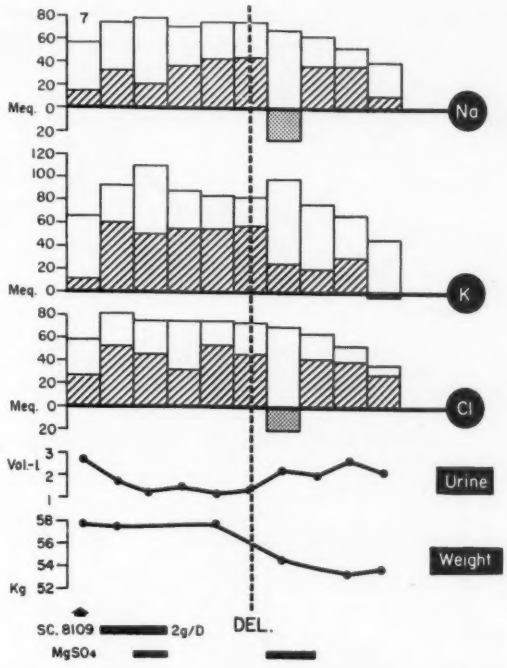

Fig. 6.—Studies of a patient in the last month of pregnancy with moderate pre-eclampsia. Short courses of SC-8109 were less effective in causing a reversal of the electrolyte balance pattern.

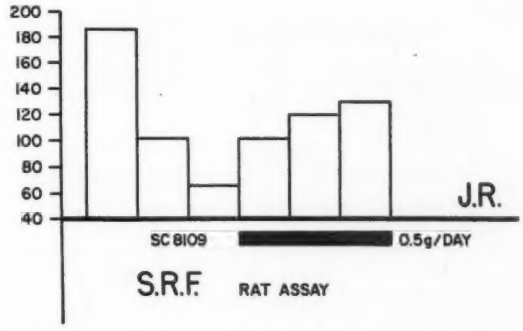

Fig. 7.—Assay results of sodium-retaining factor in a chloroform extract of the patient's urine (same patient as represented in Fig. 5). The scale on the left represents the sodium excretion of the test rat expressed as a percentage of the control rats' sodium output in the same test period. In other words, the higher the bar for each day, the less sodium-retaining material a one-hour aliquot of the patient's urine contained. The results here indicate a slight tendency of SC-8109 to depress the sodium-retaining activity of the patient's urine, but the small differences do not permit a statistical conclusion as to more than a trend.

day periods did not produce signs of endometrial atrophy in 2 other young women. As judged by the basal body temperature curve ovulation could be delayed a week by the constant consumption of 1.5 Gm. a day beginning at the conclusion of a regular period; on discontinuing the medication the patient would ovulate within 48 hours, and menstruation would then follow after a 13 day

interval. One woman maintained on 2 Gm. a day "broke through" with bleeding in 25 days, which was an anovulatory period as judged by the basal body temperature. In 3 women scheduled for therapeutic abortions daily doses in the same range for from 3 to 5 days produced no morphologic alteration in either the decidua or the placenta; in the laboratory animal, however, proportionately larger doses when started with mating or on the first day of pregnancy will interfere with implantation or the proper development of the litter.[11]

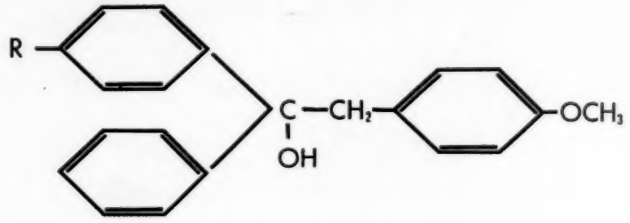

Fig. 8.—Structural formula of the antiestrogen MER-25. R is a straight chain radical, and the material is not a steroid.

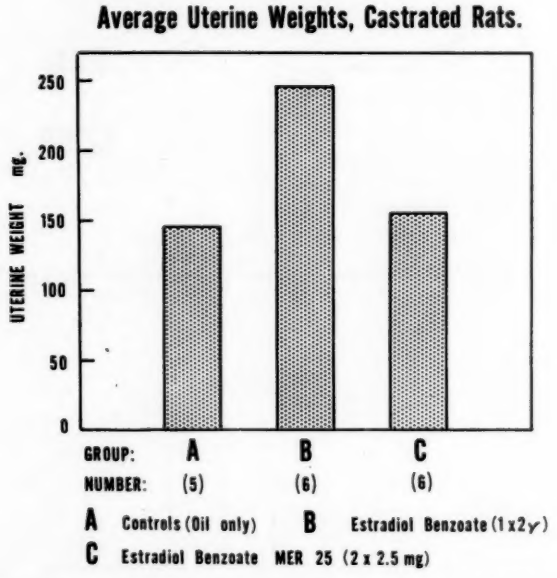

Fig. 9.—Weights of uteri of casterated rats used as an index of estrogen and antiestrogen activity. MER-25 effectively blockades the stimulating effect of estradiol.

The effect on the electrolyte and water balance of a 28-year-old regularly menstruating woman is indicated in Fig. 10. It can be seen from this figure that the administration of the antiestrogen produced an increased retention of sodium (as well as the other electrolytes studied) which would certainly not indicate any significant decline in estrogen level. The patient represented in Fig. 11 was 48 years old and 3 years past the menopause. After the first 3 days while she was achieving "balance zero" she was given 2 mg. of stilbestrol daily. For the third, fourth, and fifth days the dosage of stilbestrol was maintained at the same level but antiestrogens were also administered. Once again the 3 days that the patient was receiving antiestrogen medication were characterized by a positive balance in all of the elements studied, a balance which persisted during the

remaining 2 days when she was receiving no medications at all. Certainly from these and the other balance studies while patients were on antiestrogens it can be concluded that the estrogen effect on electrolyte excretion is not blockaded by this particular antiestrogen medication.

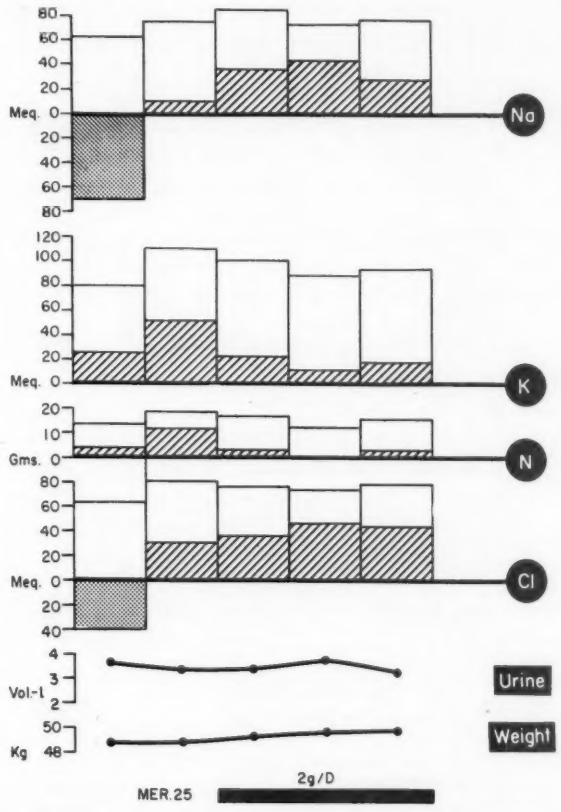

Fig. 10.—Balance studies of a regularly menstruating 28-year-old woman who received antiestrogens as indicated. The medication succeeded chiefly in increasing the retention of all elements studied.

Comment

It is entirely possible that the development of the field of the antihormones will provide therapeutic agents for the control of various clinical entities. Conditions characterized by hyperestrinism or by an excess production of aldosterone (the eclamptogenic toxemias) may yield to such an approach. In not all instances, however, does each of these agents counteract every action of the offending steroid. It will be imperative to know in each case that the particular action of the hormone which is contributing to the disease picture is successfully blockaded.

In the face of mild degrees of elevation, the anti-aldosterone examined in this report reverses the electrolyte effects of aldosterone causing increased excretion of sodium and the elimination of excess fluid, without affecting the blood pressure or other aspects of the clinical syndrome of toxemia. The antiestrogen tested, however, blockades the effect of estrogen on the vaginal epithelium and

the size of the uterus of the assay animal, but accentuates the estrogen effect on sodium metabolism and increases electrolyte retention. Precision in therapy will demand a continuation of careful evaluation of the metabolic effects of these agents.

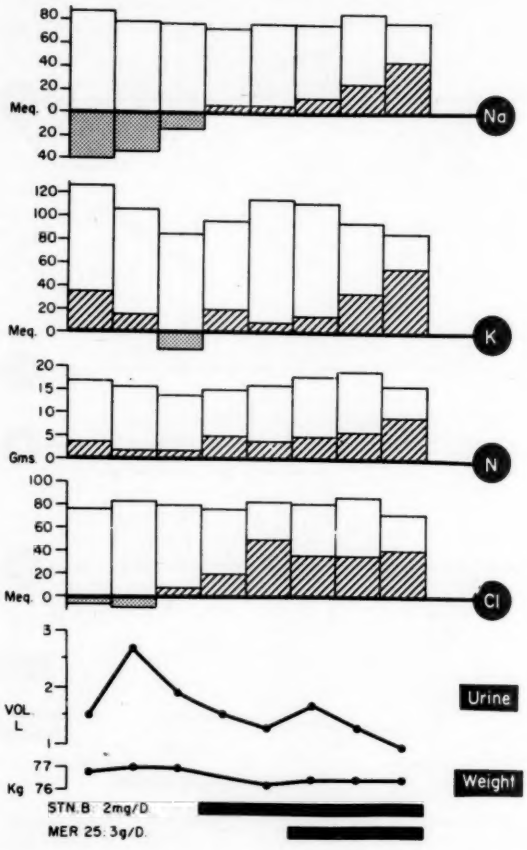

Fig. 11.—A postmenopausal patient. After an initial few days for balance adjustment, stilbestrol was administered as indicated, and then the antiestrogen added to the program. The impact of the estrogen on the balance pattern was not reversed.

Summary and Conclusions

Balance studies of water and electrolytes have been carried out in obstetric and gynecologic patients under various circumstances in which the steroid components were declining.

1. The postoperative patient demonstrates the anticipated retention of sodium with a negative balance of potassium and nitrogen. This pattern is not significantly altered by the declining estrogen level associated with oophorectomy in the regularly menstruating woman.

2. In the immediate puerperium there is a negative balance of sodium with an excretion of potassium and nitrogen that exceeds the intake. This pattern is the opposite of that seen with the administration of steroids and presumably reflects the sharp drop in these hormones.

3. The administration of an antialdosterone drug in the last trimester of normal pregnancy led to an increased elimination of sodium with retention of potassium and nitrogen, reversing the pattern attributed to the administration of aldosterone. In the patients with pre-eclampsia, however, these changes were less dramatic, possibly because an insufficient amount of medication was administered to blockade the increased aldosterone levels associated with pre-eclampsia.

4. The administration of antiestrogens either to a regularly menstruating woman or to a postmenopausal woman receiving estrogenic supplements did not reverse the electrolyte excretion pattern of the estrogens.

We would like to express our appreciation to Dr. Irving Rothchild for the animal assays of MER-25 and to Dr. James Reagan for the cornification counts. The Balance Ward could not be maintained without the efforts of Miss Beatrice Bonarrigo of the Dietary Department.

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Discussion

DR. DUNCAN E. REID, Boston, Mass.—Since all of the steroids are, to a degree involved in electrolyte regulation, the term "salt-retaining hormone" was used initially to designate the endocrine substance responsible for the conservation of salt by the renal tubule. Although hydrocortisone and cortisone have been regarded as salt retainers, this effect is offset by reason of the fact that the glomerular filtration rate is increased in the presence of these hormones. By contrast, while repeated daily intramuscular administration of estradiol produced significant fall in excretion of sodium and chloride, there was no change in urine flow, glomerular filtration rate, or renal plasma flow.[3] It is now generally accepted, however, that aldosterone is the salt-retaining hormone. Accumulated evidence from several different sources indicates that aldosterone secretion is governed not by the pituitary but possibly by the hypothalamus and more likely by the adrenal itself. The source of the hormone is apparently the granulosa layer of the adrenal cortex,[4] although in pregnancy the placenta must also be considered. In patients with complete hypophysectomy for breast cancer[4] and in one patient in whom the procedure was done during pregnancy,[7] the urinary aldosterone has remained normal. In fact, in the latter the urinary values were elevated and were consistent with those of normal pregnancy. The cortisone needs of the pregnant patient were identical with those of the nonpregnant patients, approximately 75 mg. daily.[7] This clearly indicates that the placenta is not secreting adrenal-like corticoids in anything resembling near physiologic amounts. Aside from chorionic gonadotropin, the sex steroids, and their intermediates, it is questionable whether the placenta produces other hormones.

It is also uncertain how the adrenal secretion of aldosterone is regulated. Two theories have emerged. First, aldosterone secretion is influenced by the body's need for sodium. Urinary aldosterone, which presumably reflects the amount of aldosterone being secreted, is high when sodium is being retained, as observed in the normal individual deprived of

sodium or when there is a pathologic retention of sodium as in congestive heart failure or nephrosis. Second, aldosterone secretion is altered by changes in the extracellular fluid volume regardless or independently of sodium.[2-6] When there is water loading, urinary aldosterone levels fall. More impressive has been the observation that in diabetes insipidus either primary or secondary to complete hypophysectomy, urinary aldosterone levels are maximum when the urine volume is greatest and the extracellular fluid volume is presumably least. The urinary levels of aldosterone fall with the decrease in urine volume following Pitressin administration.[2, 4, 7] This suggests that extracellular volumetric changes can regulate aldosterone secretion as well as the body's need for sodium.

To the discusser at least, pregnancy seems paradoxical to both theories unless one assumes that this normal situation is comparable to that present in patients with congestive heart failure or that the pregnant woman is retaining salt in excess of her needs. As Dr. Barnes and others have shown, there is a four- to eightfold increase in urinary aldosterone in normal pregnancy.[1, 9, 10] Thus, pregnancy represents a unique situation with an expanding extracellular fluid volume and sodium retention in the presence of a high urinary aldosterone excretion. If this is correct, I should like to ask Dr. Barnes whether he has any working hypothesis as to the cause-and-effect relationship between the urinary aldosterone values and the increase in extracellular fluid volume in pregnancy. Further, Dr. Barnes, do you believe that urinary aldosterone levels are an index of utilization of the hormone or do they simply reflect secretion, excretion, or both?

Aldosterone antagonists compete for sodium, which is reflected by a marked increase in urinary sodium and a disappearance of urinary aldosterone. There can be no doubt that Dr. Barnes and his group have demonstrated that following administration of one of these aldosterone antagonists to a normal pregnant patient there was a remarkable urinary sodium loss. He is naturally disappointed, as indeed are all clinicians, that this response was much less in patients with toxemia but, as he states, it may still be a matter of dosage for unfortunately the toxicity of these substances apparently allows for only short periods of administration.

As Dr. Barnes has demonstrated, the postdelivery sodium loss is considerable and this, together with the fact that the water turnover rate is accentuated in the early puerperium, accounts for the postpartum diuresis. Several years ago Dr. Howard Taylor and his group were able to delay this phenomenon for a few days by the administration of sex steroids. Is it Dr. Barnes's feeling that the electrolyte loss following delivery is the result of a sudden decrease in sex steroids or a decrease in adrenal activity? Were it the latter, this would be akin to an adrenal crisis, hardly in keeping with the clinical state of the seemingly normal postpartum patient.

Undoubtedly all would agree that a knowledge of the factors responsible for the control of extracellular fluid during pregnancy would afford more insight into the physiology of reproduction than any other body of information. It would also afford an insight into the medical conditions associated with deviations in normal water and electrolyte balance. If Dr. Barnes's reported findings can be confirmed, he is to be congratulated for he has initially demonstrated that the electrolyte pattern in pregnancy can be changed by alterations of the endocrine factors.

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DR. RUSSELL R. de ALVAREZ, Seattle, Wash.—These carefully executed metabolic balance studies by Dr. Barnes offer convincing findings of nitrogen, fluid, and electrolyte balance in the normal patient, as well as in patients in various states of stress. His findings of nitrogen, sodium, and potassium balances in patients with pre-eclampsia coincide almost exactly with those reported by us. It is logical that, inasmuch as the effects of the so-called antisteroid preparations have been demonstrated in animals, their effectiveness can be determined in humans. This Dr. Barnes has attempted to do. Even though the antiestrogen is nonsteroidal and the antialdosterone is steroidal, the mechanism of action does not seem to be a direct action on the ovarian or adrenal steroids themselves but one of blocking their effectiveness. In the castrated experimental animal, antiestrogen inhibits increases in uterine weight and the production of vaginal cornification. The term "antialdosterone" is applied to the reversal of the electrolyte effects produced by the adrenal cortical hormones. Since desoxycorticosterone also increases tubular reabsorption of sodium even though not to the degree evoked by aldosterone and, since the antialdosterone preparations also inhibit the action of DCA, it seems semantically appropriate to designate these effects as antimineralocorticoidal.

The concept of analogues in competitive inhibition was originally proposed in relation to serotonin by Wooley. It seems that the so-called antisteroid analogues act in competitive inhibition on the cell in much the same manner. Just as 19-nor analogues of some of the newer progesterone preparations exhibit markedly increased progestational activity, so too does the 19-nor analogue of the antialdosterone studied by the authors seem to be a more powerful blocker of the electrolyte effects than if the 19-R group were retained.

Aldosterone occurs in two forms. One form is known to possess activity in the body, while the physiologic activity of the other has not yet been demonstrated. The active form of aldosterone in the body is the hemiacetal form and is represented by the rearrangement of the hydroxyl group at the C_{11} position in conjunction with the C_{18} position. Dr. Barnes stated that the excretion of aldosterone in his studies was measured by the assay of adrenalectomized rats by the method of Kagawa. We have not been able to obtain satisfactory results utilizing the bioassay method of measurement of aldosteronuria. We have, however, been obtaining consistently satisfactory results utilizing three paper chromatographic systems. The data presented by the essayist are confined to metabolic balances of nitrogen and electrolytes. It seems that significant aldosteronuria would be inhibited by some of the relatively large intakes of sodium allowed so that even if the antialdosterone were an effective blocker there would seem to be relatively small amounts of aldosterone on which the antisteroid might act. Inasmuch as statements were made about the measurement of aldosterone, it would be interesting and helpful to have information regarding the quantitative measurements of aldosterone excretion and to know whether any shifts in aldosteronuria occurred under the influence of the agent.

Studies of the pre-eclamptic patient represented by Fig. 4 showed a sustained positive sodium balance during the administration of the antialdosterone. Rather than applying the explanation for the sharp contrast in sodium balance as being due to inadequate dosage administered to the patients represented by Figs. 5 and 6, one must consider the fact that in one of these individuals pre-eclampsia was present while it was not present in the other. Even though the renal sodium load was similar in both instances, the renal tubular handling of sodium is different in the pre-eclamptic patient as compared with the normally pregnant patient.

Dr. Barnes's studies of these antisteroid agents show discouraging results by the methodology utilized in that not only was there no reversal of electrolyte excretion, but also no significant catabolic process occurred as measured by nitrogen balance. It is interesting

to note that Fig. 8, the structural formula for antiestrogen, appears very similar to the formula for TACE. The basic carbons of the antiestrogen preparation are saturated when compared to the formula for diethylstilbestrol. The postmenopausal patient represented in Fig. 11 received stilbestrol following which the antiestrogen was added. Based on the similarity of the formula of TACE and the antiestrogen used, it would be interesting to speculate whether Dr. Barnes would have received the antiTACE effect instead of attempting to produce an antiDES effect.

While the exact action of antihormones is not known even in the experimental animal, it would seem that an antihormone most likely should possess an effect counteracting the hormone itself. Whether counteracting the effect of hormone stimulation by antihormones is truly an effect against the initiating hormone is not yet known. Perhaps the antihormones may be capable of altering protein synthesis or stimulating immunity responses so that their activity evokes an antigen-antibody response.

DR. BARNES (Closing).—Dr. de Alvarez asks if output measurements of aldosterone per se have been carried out. No. The measurements cited are on sodium-retaining components excreted in the urine. I think his explanation of the possible failure to reverse the electrolyte pattern in pre-eclampsia is valid. One must simply leave the door open for the possibility that the pre-eclamptic patient can absorb more antialdosterone than can the nontoxemic pregnant woman. His comment on the end point which is selected to define an antiestrogen is valid, and is the very point we were making. With the electrolyte effect as the end point, I do not think that this is truly an antiestrogen.

Dr. Reid asks the specific question as to the increase in volume of extracellular fluid in the pregnant patient where there is apparent disregard for this in so far as her own hormone production is concerned. This is, of course, true, and is one of the phenomena of pregnancy that interests us. The pregnant woman does have an increased extracellular fluid volume and her control mechanisms behave as if they did not know this.

Dr. Reid asks if I feel that this is representative of a decline in ovarian steroids that are dropping sharply or a decline in corticosteroid level as the adrenal ceases its hyperactivity of pregnancy. I have no way of sorting out which factor is which, but I am convinced that the control of water and electrolyte balance is panglandular so I believe the answer would be both: all the steroid components which are declining have their influence on this pattern.

THE HEMODYNAMICS OF A UTERINE CONTRACTION*†

CHARLES H. HENDRICKS, M.D., CLEVELAND, OHIO

(From the Department of Obstetrics and Gynecology, Western Reserve University School of Medicine)

THE uterine contraction is the basic unit of labor. Since labor is built largely on these contractions, we need to learn as much as possible about their physiology if we wish to increase our understanding of the process of human parturition.

Various components must contribute to uterine contractility: muscular, nervous, hormonal, enzymatic, and vascular. The present report is concerned with the *vascular* aspects of the uterine contraction.

When the pregnant uterus contracts, a substantial amount of blood is pushed out into the systemic circulation.[1] There is evidence[2] that in a vigorous contraction during labor the cardiac output rises a great deal, averaging in some moment an increase of about 30 per cent over the lowest output present during the uterine resting phase. It should be apparent that only a fraction of this rise is attributable to the amount of blood extruded from the uterus, because during active labor there are present other factors which also tend acutely to elevate the cardiac output. Quantitatively, the most important of these factors are pain, anxiety, and nonuterine muscular activity. In other words, the previously described augmentation in output during uterine contractions represents the total response to a complex of factors.

The volume of blood extruded from the contracting uterus and presented to the central circulation for disposition (a process which we term "volume redistribution") makes a relatively small but nevertheless highly significant contribution to the augmented cardiac output. If volume redistribution is to be studied as an "isolated" phenomenon, one must eliminate in so far as possible all the other known factors which tend to raise the cardiac output during uterine contraction.

Most of the studies herein reported were carried out at the Sección Fisiología Obstétrica, the laboratory of Drs. Hermongenez Alvarez and Roberto Caldeyro-Barcia, at the Facultad de Medicina, Montevideo, coincident with uterine contractility evaluations which were being performed in pregnant subjects. The studies were aided in major degree by the helpful advice and warm cooperation of Drs. Caldeyro-Barcia and Alvarez and their staff.

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Methodology

1. Conditions.—It became evident early in the study that any small disturbance would alter and confuse the results significantly. Therefore, except when otherwise noted in the text, serious interpretation of the records was attempted only when the laboratory was silent, and when it was felt that the patient could be kept under conditions in as nearly a steady state as possible under the existing circumstances. The patient had to be quiet, relaxed, and without significant pain during the uterine contractions. If the contractions were severe enough to cause pain, medication (Demerol and/or chlorpromazine) was given until the patient was free of pain before readings were resumed. Any physical motion by the patient or the fetus was recorded on the continuous record. Fetal motion was observable both directly and also in the record of amniotic fluid pressure.

2. Recording.—All phenomena under study were recorded simultaneously on a Sanborn Polyviso, at rates varying from 0.5 cm. to 200 cm. per minute, depending upon the information which was being sought. The factors observed included:

A. Intra-amniotic pressure obtained by the method of Alvarez and Caldeyro.[3]

B. Intra-arterial pressure recorded continuously, with the use of a long thin polyethylene (1 mm. OD) catheter in a femoral artery. Several brachial arterial recordings were also made.

C. Central venous pressure was estimated by insertion of a polyethylene catheter at least 12 cm. inside the right anterior axillary line. Brachial venous and femoral venous pressures also were recorded.

3. Estimation of Cardiac Output.—This was done by the blood pressure method, as modified by Remington and associates,[4] and as previously utilized by Hendricks and Quilligan.[5] Output estimations were made from tracings taken at high speeds, and determinations were made at 6 second intervals, the pulse rate and pressures of virtually *all* beats being taken into consideration for establishing an estimate of the average total cardiac output during each 1/10 minute.

4. Method for Estimating Volume Redistribution.—Since under such "steady state" conditions of labor the cardiac output is fairly stable between contractions, it may be fair to assume that in the absence of pain, anxiety, or effort any increase in cardiac output associated with the onset of a uterine contraction is due to extra blood presented to the central circulation for redistribution by the contracting uterus. Since the cardiac output is being calculated in "blocks of time" of 1/10 minute each, any increase in the *rate* of cardiac output can be translated into the number of additional cubic centimeters of output over the base line output in that 6 second period.

For example, if the indicated cardiac output in uterine diastole is 6.00 L. per minute, and the rate rises during a given 6 second period to 6.50 L. per minute, this indicates that during a minute of flow at the higher rate an additional 500 c.c. of blood would be pumped out by the heart; thus, in 6 seconds (or 1/10 minute), the additional cardiac output would be 50 c.c. The sum of these increments above the steady state level constitutes an estimate of the total additional amount of blood put out during that time, and may be interpreted as being equivalent to the amount of blood extruded from the uterus during the contractile phase.

While the limitations of the method are recognized, the results are consistent, and fit well into a logical pattern of over-all cardiovascular response to the contracting uterus.

Results

1. Volume Redistribution.—Volume redistribution was estimated by the method described above. The results for 27 contractions are listed in Table I. It may be seen that the average amount of blood redistributed during an effective uterine contraction was 296.0 c.c., with a range from 176 to 448 c.c. It was found that the amount of blood redistributed during a contraction did not bear any particular relationship to the peak intra-amniotic fluid pressure as long as the intra-amniotic pressure rose at least 20 mm. Hg above the tonus. The only observed exception to this rule was that under conditions of hypertonus there was no consistent evidence that redistribution was occurring (Table II). This is considered a significant point, as pointed out in the discussion below. In contractions where a maximum intra-amniotic fluid pressure of 20 mm. Hg was not attained, there was no consistent evidence of blood redistribution.

TABLE I. VOLUME REDISTRIBUTION, ADEQUATE CONTRACTIONS, NORMAL TONUS

IDENTIFICATION	POSTURE	OXYTOCIN INFUSION (mU*/MIN.)	TONUS (MM. HG)	MAXIMUM AMNIOTIC FLUID PRESSURE (MM. HG)	VOLUME REDISTRIBUTION (C.C.)	TIME OF VOLUME REDISTRIBUTION (SECONDS)
578 a	Supine	0	9	54	258	42
578 b	Supine	0	7	30	179	42
578 c	Supine	0	8	28	282	42
578 d	Supine	0	8	56	320	48
578 e	Supine	0	9	39	176	48
678 c	Supine	0	5	49	269	42
678 b	Supine	0	9	74	240	60
682 a	Supine	0	5	26	202	36
682 b	Supine	1	4	24	259	36
682 c	Supine	1	4	36	230	54
682 e	Supine	0	3	36	288	42
682 f	Supine	2	5	57	250	36
682 g	Supine	2	5	54	448	42
682 i	Supine	16	8	66	400	54
682 j	Supine	16	4	66	256	24
682 k	Supine	16	5	60	384	30
685 a	Supine	1	5	63	325	30
685 b	Supine	1	7	60	406	48
685 c	Supine	1	8	52	310	24
678 e	Supine	0	4	68	298	30
681 a	Supine	0	8	28	426	42
681 b	Supine	0	8	39	302	42
681 c	Supine	0	7	53	344	30
681 d	Supine	2	4	38	256	36
678 a	Left side	1	8	51	365	72
682 d	Right side	2	5	35	280	24
682 h	Right side	8	7	35	240	60
Mean			6.2	47.3	296.04	41.3

*mU = Milliunits.

Fig. 1 represents graphically the times during which the cardiac output was elevated above the base line, and also the time when the maximum increase in cardiac output occurred. It may be seen that the time required for redistribution varied from 24 to 72 seconds (average 41.6 seconds). The maximum peak of redistribution appeared approximately 18 seconds, on the average, before the peak amniotic fluid pressure was attained.

TABLE II. EFFECT OF HYPERTONUS ON VOLUME REDISTRIBUTION

IDENTIFICATION	POSTURE	OXYTOCIN INFUSION (MU/MIN.)	UTERINE TONUS (MM. HG)	MAXIMUM AMNIOTIC FLUID (MM. HG)	VOLUME REDISTRIBUTION (C.C.)
678 J	Supine	1	17	45	−168
678 H	Supine	1	14	34	101
678 i	Supine	1	13	59	56
678 j	Supine	1	13	47	45
678 k	Supine	1	14	41	130
678 l	Supine	1	11	32	115
678 m	Left side	1	20	61	64
682 l	Supine	2	13	24	−101
678 n	Supine		12	58	−141
681 e	Supine	0	13	67	+187
681 f	Supine	0	15	57	+368
Mean			14.1	48.7	131.1

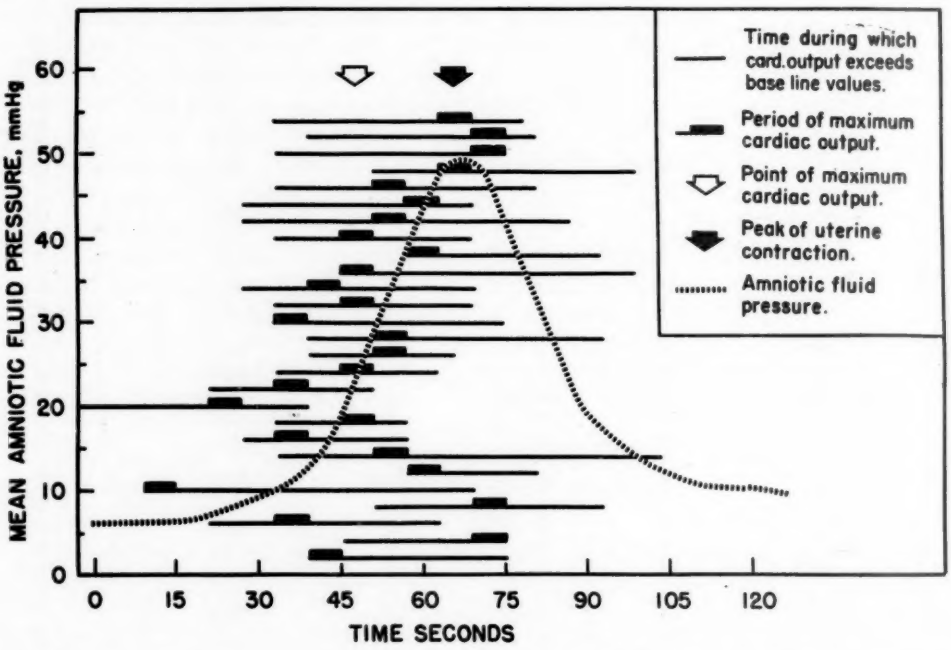


Fig. 1.—Shows the relationship between the state of uterine contractility and changes in cardiac output, as observed in 27 contraction cycles. The time during which the output is elevated above the base line is represented by the solid horizontal lines, while the period of maximum cardiac output for each contraction cycle is represented by the small black rectangle above the horizontal line. The dotted line is a composite of the mean amniotic fluid pressure for this group of contractions. The average point of maximum cardiac output occurs about 18 seconds before the peak of the amniotic fluid pressure is reached.

Fig. 2 shows the mean values for 20 contraction cycles. The curved line is drawn from the average intra-amniotic fluid pressures. The other line represents cardiac output values taken at 6 second intervals. Determinations were carried out for 1 minute periods before and after the apex of the uterine contraction. All values were corrected from cardiac index to cardiac output by use of the patient's body surface values.

It was of great interest to note that in most cases the increase in cardiac output began very early, actually long before a patient could possibly be aware

that a contraction was under way. When the mean amniotic fluid pressure had risen by only approximately 3 mm. Hg above the tonus level, there was already a rather marked rise in cardiac output, and this rise advanced progressively during the ensuing 24 seconds. It reached its apex approximately 18 seconds before the apex of the contraction, and thereafter declined progressively. For the 20 contractions included in this figure, 86 per cent of the mean increase in cardiac output over the base line level had already been redistributed by the time the peak amniotic fluid pressure was reached.

This pattern of redistribution was found to be identical whether the contractions were entirely spontaneous or whether they were the result of constantly administered intravenous synthetic oxytocin. The pattern was most consistent when the patient had been stabilized in the supine position. The patient in the lateral position often showed no predictable response.

It was noted repeatedly that the indicated cardiac output changes, when the subject was undergoing direct physical effort, anxiety, fright, or pain, were so great as to mask completely the redistribution pattern. Any direct physical

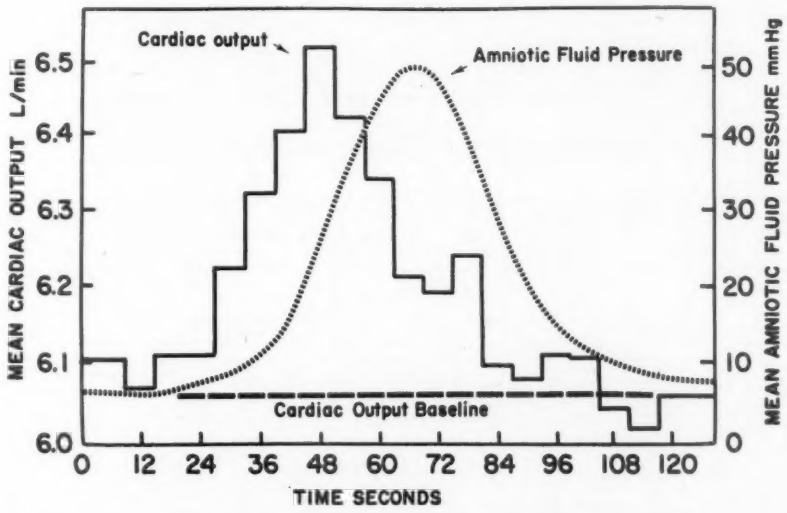


Fig. 2.—Alteration in cardiac output during uterine contraction. The mean cardiac output (as indicated by the pulse pressure method) for 20 complete contraction cycles shows a rise extremely early in the contraction, and reaches an apex about 18 seconds before the maximum amniotic fluid pressure is attained.

effort on the part of the patient, for example, would alter the cardiac output more or less in proportion to the effort involved. On one occasion when a subject was startled by the dropping of a bedpan, the cardiac output rose by nearly 500 c.c. in a very short period of time, and thus this "startle reaction" also would serve to mask the phenomenon described in this study. A patient in late labor who is experiencing a combination of disturbing factors was found to have an increase in her indicated cardiac output during a contraction cycle as much as 2,500 c.c. Thus it becomes evident that the phenomenon of redistribution cannot be successfully measured when fright, pain, or physical effort is present. It was gratifying to note, however, that even in late labor where ideal laboratory conditions prevailed and where the patient was entirely pain free, the redistribution pattern remained essentially constant both as to the time of redistribution and the amount of blood redistributed.

2. Blood Pressure.—It is an old observation that the blood pressure tends to rise during the active part of a uterine contraction, but the details of this alteration have remained incompletely documented. From the results of this study it

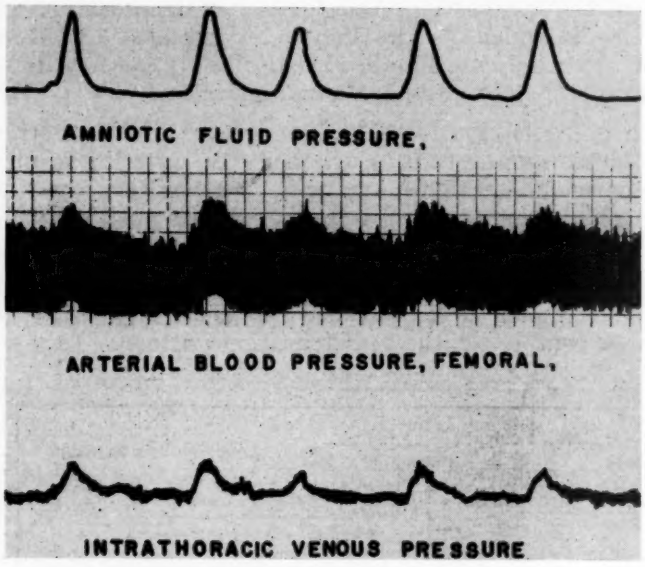


Fig. 3.—Shows the close relationship between uterine contractions and the arterial blood pressure. The arterial blood pressure rises from 15 to 20 mm. Hg during the time the uterine contraction is developing, dropping slowly as the contraction subsides. The intrathoracic venous pressure follows somewhat the same pattern, but reaches a peak somewhat earlier, and subsides somewhat more rapidly. Time scale: 2 of the small squares per minute.

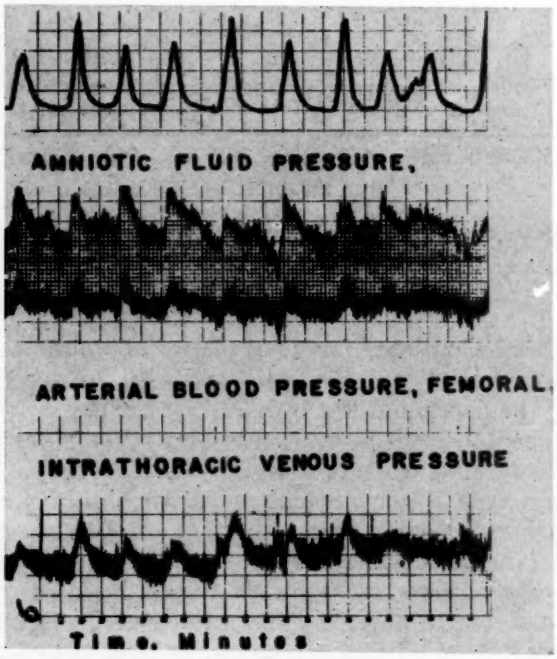


Fig. 4.—An exaggerated blood pressure response to uterine contractions. The rise in blood pressure is from 20 to 30 mm. Hg, and the maximum pressure is attained before the apex of the contraction is reached.

has become apparent that a fairly definite rise in blood pressure, usually of the range of 10 to 20 mm. Hg, does occur with many contraction patterns. For example, Fig. 3 shows a tracing of a more or less typical pattern wherein the blood pressure rises by 15 to 20 mm. with each contraction. Fig. 4 shows an exaggerated response, where the rise approaches 30 mm. Hg in some instances.

Fig. 5 illustrates the mean variations in blood pressure during 20 uterine contraction cycles. From a study of this figure it may be noted that the diastolic pressure response is relatively small, a rise of only about 5 mm. Hg occurring during the active contractile phase. In the systolic pressure, however, the average rise is closer to 10 mm. Hg. Thus it is seen that the net effect of the blood pressure alteration is to increase the systolic pressure far more than the diastolic pressure, thus bringing about a significant increase in the stroke during

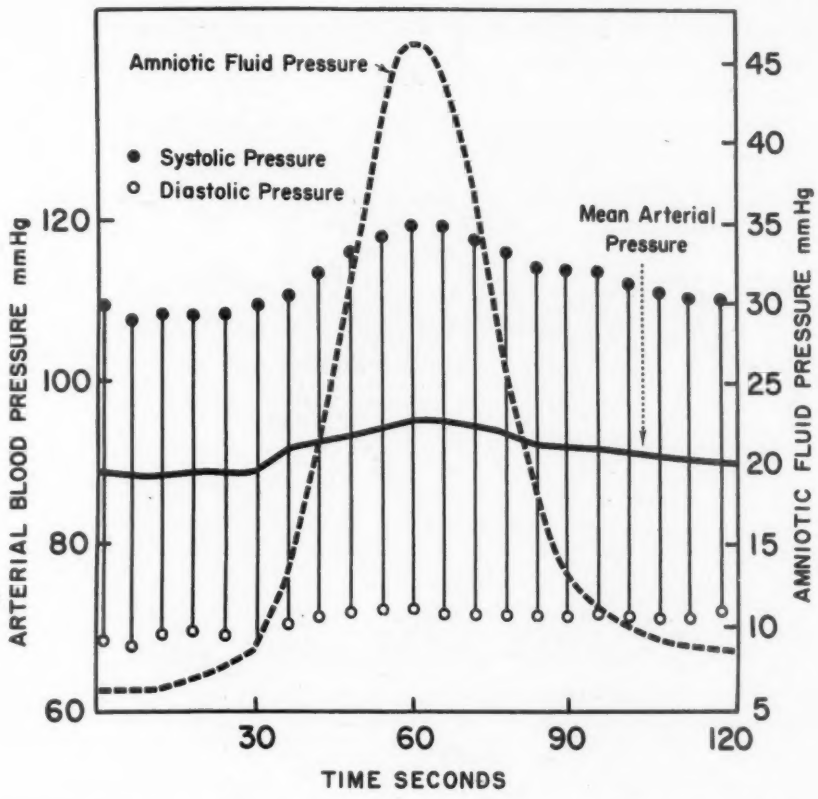


Fig. 5.—Variations in blood pressure during uterine contraction. Mean values, 20 complete contraction cycles. Although there is wide variability in the degree of blood pressure response during uterine contractions, there is a consistent tendency toward elevation of the pressure, with the systolic rise substantially exceeding the diastolic rise.

the contractile phase. The *mean arterial pressure* rise is of course smaller than the rise in the systolic pressure, because of the fact that the increase in the diastolic pressure is less than the increase in the systolic pressure.

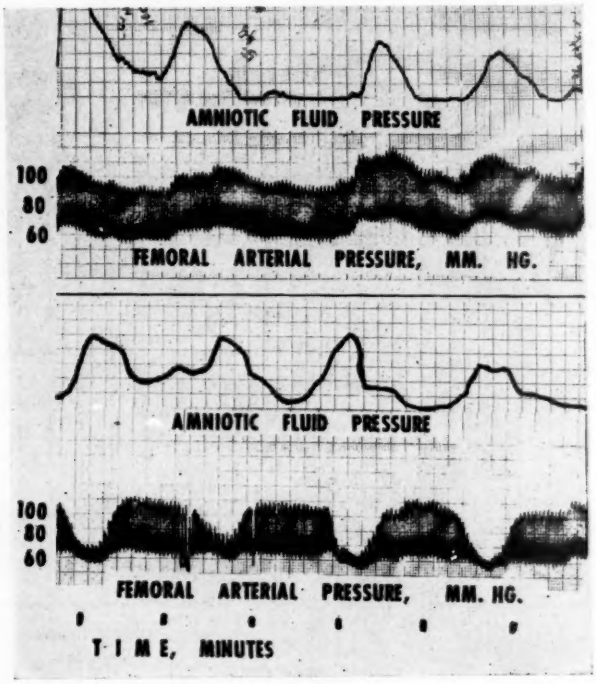
It should be pointed out, however, that in some subjects such pressure rises do not appear during contractions. Furthermore, in other subjects in whom such alterations have been observed, the change may decrease or increase spontaneously at a later time in labor. The one factor which appears most consistently to affect the blood pressure rise is that of posture, inasmuch as often when a subject shifts from the supine to the lateral position the pressor response

to the uterine contraction disappears. The disappearance or alteration of this response also may in some cases apparently be brought about simply by a slight shift in the position of the fetus.

When the femoral arterial pressure is being recorded, the onset of a uterine contraction may under certain postural conditions bring about an actual *decrease* in the recorded pressure. This phenomenon was observed in 1955 by Dr. J. J. Poseiro,[6] working at the Seccion Fisiologia Obstetrica, in Montevideo.

Such a case was also recorded in our own laboratory. At the top of Fig. 6, it may be seen that when the subject was in the supine position there was a consistent rise in the right femoral arterial pressure during the contractions. When the subject was shifted toward the right side, however, there occurred a drastic drop in the recorded pressure. Since there was no evidence that the catheter was being obstructed in any fashion, it is felt that the decreased pressure most likely resulted from pressure upon the femoral arterial supply brought about either directly or indirectly by the uterine contraction.

A.



B.

Fig. 6.—While the subject was supine, the blood pressure rose consistently during uterine contractions. When the subject was turned to the right side, however, there was observed a drop in the recorded femoral arterial pressure in response to uterine contractions. There was no evidence of obstruction of the catheter. This phenomenon may be observed intermittently in the same subject, but is usually difficult to reproduce deliberately. *A*, Supine position. *B*, On right side.

3. Heart Rate.—The rate response was found to vary considerably from one subject to another, and sometimes even from one contraction to another in the same subject. The general pattern, however, is illustrated by the curve in Fig. 7, which represents the mean values of 20 contraction cycles. It is observed that as a contraction develops there is initially a very small rise in heart rate followed by a significant drop which approximates 12 per cent below the heart rate at the resting level. As the uterus then relaxes there is a slow return to the original resting heart rate.

4. Stroke.—The stroke, at least as indicated by the pulse pressure method, appears to vary quite consistently in the opposite direction from variation in the heart rate. Initially there appears to be a small drop in the stroke (Fig. 7), and then as the contraction develops there is a rise which approximates 17 per cent above the resting phase. As the contraction subsides, the stroke volume again drops progressively toward the resting level. The more or less reciprocal nature of the variations of stroke and heart rate as illustrated in Fig. 7 is a matter of great interest. For example, if either the stroke or the heart rate remained constant while the opposite function varied as shown in Fig. 7, there would be fairly large alterations in cardiac output resulting from the uterine contraction. The fact that the actual change in cardiac output during a contraction cycle appears to average less than 300 c.c. furnishes a good example of how, through compensating alterations in either stroke or heart rate, the cardiac output may be maintained at a relatively constant level, varying only in response to alteration in physiological conditions. It should be pointed out, however, that in spite of the excellent compensation observed we do not have an adequate explanation as to why either the rate *or* stroke would vary by such large amounts during such a large portion of the contraction cycle.

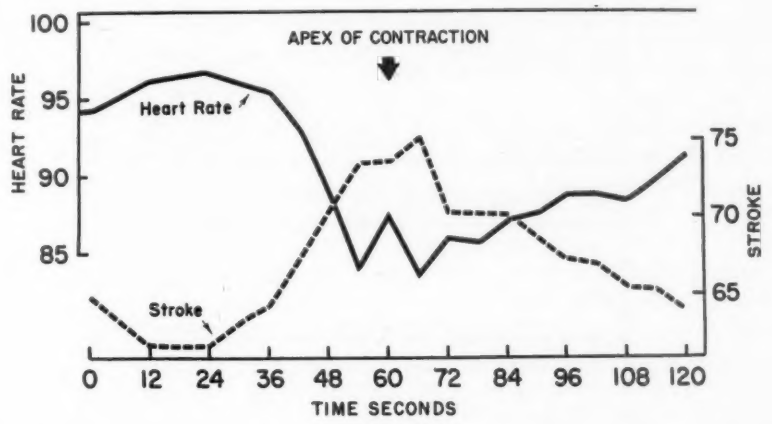


Fig. 7.—Variations in heart rate and stroke during uterine contraction. The mean values of the stroke (as indicated by the pulse pressure method) and of the heart rate were determined at 6 second intervals for 20 complete contraction cycles. There may be seen a rather consistently reciprocal relationship between these two factors, which serves to maintain the cardiac output at a fairly steady level. The greatest departure from an even cardiac output comes early in the contraction, when there is a mild increase, which is interpreted as being due to redistribution of the blood extruded from the uterus at the onset of a contraction (see Fig. 2).

5. Venous Pressure Changes.—There were found to be rather consistent elevations in the central venous pressure induced by uterine contractions. In most instances (Figs. 3, 4, and 8) the rise of 4 to 6 mm. Hg begins early in the contraction cycle, reaches a peak by the time the contraction is at its maximum, and then subsides progressively to the original level. There seems to be some correlation between the amount of blood pressure rise and the amount of rise in the central venous pressure. Under conditions of relatively high uterine tonus there appeared to be little change in the central venous pressure.

In Fig. 8, *A*, the intrathoracic venous pressure was recorded at a speed of 30 cm. per minute, while in 8, *B*, *C*, and *D*, the recording speed was 3 cm. per minute. The rise in central venous pressure is fairly representative. Unfortunately, this subject was experiencing some discomfort with her contractions, so that there is some possibility that all the rise is not due solely to the contraction.

It is interesting to note that in the same subject and under essentially the same conditions, the brachial venous pressure (Fig. 8, *C*) showed little or no response to uterine contractions.

The venous pressure response in the femoral area, however, is a different matter. Fig. 8, *D* is a tracing taken from a different subject than the subject used in Fig. 8, *A*, *B*, and *C*. This subject was supine, and resting quietly under spinal anesthesia. During the *very early* portion of the contraction, there was a sharp rise of femoral venous pressure, increasing by 20 mm. Hg in some instances. By the time the amniotic fluid pressure had reached its apex, the venous pressure had dropped about halfway back to the base line level, and this "plateau" of pressure was then maintained until the contraction had subsided almost completely.

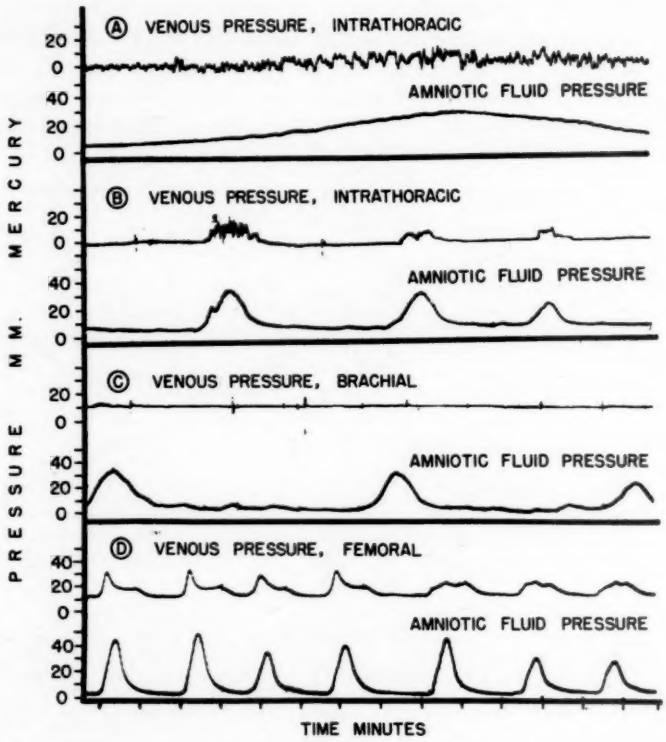


Fig. 8.—*A*, Intrathoracic venous pressure; response to uterine contraction (speed of record: 30 cm. per minute). *B*, Intrathoracic venous pressure, recorded at 3 cm. per minute. The pressure rises significantly in association with the increase in amniotic fluid pressure. *C*, The brachial venous pressure shows little or no alteration in response to the uterine contractions. *D*, The femoral venous pressure rises sharply by as much as 20 mm. Hg during the early part of the contraction cycle, then drops to a "plateau level" until the completion of the active part of the contraction, when it subsides further to the base line (noncontractile) level.

Comment

Various factors involved in the cardiovascular response to uterine contractions have been mentioned in the literature, usually appearing somewhat incidentally in the course of reports on the cardiovascular changes of pregnancy in general. The blood pressure rise has been mentioned by many, including Jensen,[7] and the physiology of the phenomenon was discussed in more detail by Woodbury, Hamilton, and Torpin.[8] The latter authors reported that the pulse pressure widens during a contraction, while Haupt[9] believed that the pulse stroke

volume shows a decrease with contractions, but that this decrease is not constant. Palmer and Walker[10] reported a rise in right atrial pressure with a contraction. Perhaps the greatest amount of comment in the literature concerns the pulse rate. Martin[11] found in 1854 that the pulse rate increases with pains, returning to normal levels between pains in early labor, but remaining elevated in late labor. Pardee and Mendelson[12] observed that a uterine contraction brings about a slight increase in pulse rate. Twitchell, working in Burwell's[13] laboratory found a rise in heart rate during the early seconds of a uterine contraction, followed by a marked slowing during the time when the contraction was most intense, a pattern which corresponds to the findings in the current report.

These studies vary widely in study methods and conditions. Also, with the exception of a few recent workers, such as Burwell and Woodbury, Hamilton, and Torpin, there has been little recognition of the fact that any of the parameters may vary widely according to the phase of the contraction.

The following paragraph is an attempt to tie together an explanation of circulatory events as they appear to occur solely in response to a contraction when the subject is under the most favorable study conditions, i.e., when the disturbing influences of pain, apprehension, physical motion, and general anesthesia have been eliminated. It is recognized that the response of any one parameter may vary widely from one individual to another, not only because of the variability of a single phenomenon from one individual to another, but also because the variability in a single factor—the heart rate, for instance—may be modified by the interplay of other factors which are also operating to maintain "circulatory compensatory adjustment."

The maternal hemodynamic response to a uterine contraction follows an orderly and predictable sequence, which may be explained as follows. The beginning of an effective uterine contraction appears to push a fairly large component of blood (probably in the range of 250 to 300 c.c.) rapidly into the maternal central venous reservoir. This results in a rapid rise in the central venous pressure, but this rise is not shared by the peripheral venous system in the upper extremity. In the lower extremity, however, there may be a sharp transient rise in venous pressure at the very beginning of the contraction, at the time when venous blood from the lower extremities is "competing" with that being flushed from the uterus for entry into the maternal central venous reservoir. Even though the sharp peak of femoral venous pressure is not sustained, there remains a modest elevation of femoral venous pressure until the uterus relaxes. The first response of the heart to the increased central venous load is to increase the heart rate; but as the blood pressure rises, the stroke increases, and the heart rate drops even below that of the normal resting rate. When redistribution has been accomplished, all parameters return to their original base line levels until the onset of the ensuing contraction.

Opinion has differed in the past as to whether, during a uterine contraction, (a) the blood from the uterus is squeezed out, or (b) whether most of the uterine blood, and particularly that in the intervillous space, is "trapped" inside the uterus.[14] It is not the purpose of this paper to claim that all of the intrauterine blood is squeezed out when the uterus contracts. Such a claim would be most unrealistic. Although the actual volume of blood contained in the pregnant human uterus at term has never actively been determined, evidence obtained from animal experimentation[15] indicates that 500 to 600 c.c. of blood would be a reasonable value, although at least one group of workers have thought the true value might be as high as 1 L.[16] As the uterus begins to contract, the increased myometrial pressure serves to squeeze any available blood back into the central venous circulation. The same increased pressure, however, very soon collapses the intramyometrial veins.[17] A certain volume of blood then may well

be relatively immobilized within the intervillous space, and this amount of blood may conceivably be further augmented by continued arterial flow until arterial circulation itself is severely limited by the increasing myometrial pressure. It appears then that perhaps both of the classical theories may be partially correct, part of the uterine blood volume being extruded to the general circulation and the remainder being temporarily held within the maternal subchorionic lake. While the present study offers no direct support for the "trapping" theory, it may be pointed out that the results obtained cannot possibly account for all of the uterine blood volume solely on the basis of systemic redistribution; and because of the dramatic increase in intramyometrial pressure it is a reasonable assumption that a significant component of maternal blood collects or remains at the placental site.

The failure of any consistent increase in the indicated cardiac output under conditions of uterine hypotonus (Table II) is probably to be anticipated. With abnormally high tonus, there is less opportunity for the full component of venous blood to be extruded from the uterus, because the increasing pressure on the myometrial veins more rapidly approaches the point at which uterine outflow is occluded.

Summary

1. An attempt has been made to estimate by indirect methods the amount of blood extruded from the uterus into the maternal venous reservoir during the early portion of the contraction cycle. From this study it would appear that this volume of blood may be in the range of 250 to 300 c.c.

2. The blood pressure rises quite consistently during a uterine contraction. Most commonly, the systolic pressure rises by 10 to 20 mm. Hg, while the rise in diastolic pressure is somewhat less.

3. During the early part of the contraction the heart rate tends to rise, followed by a substantial lowering of the rate by the time the contraction is at its maximum intensity.

4. The stroke volume appears to drop slightly during the initial stages of the contraction cycle, after which it rises significantly above the base line level.

5. The heart rate and stroke appear to maintain a somewhat reciprocal relationship throughout the contraction cycle.

6. Central venous pressure rises in response to a uterine contraction. Brachial venous pressure shows little or no response. Femoral venous pressure rises sharply in the very earliest phase of the contraction, and then drops to a somewhat lower elevation for the rest of the active portion of the contraction, after which it subsides to its original level.

In addition to expressing gratitude for the enthusiastic cooperation afforded by the other members of the Sección Fisiología Obstétrica, I would like especially to acknowledge my indebtedness to the Sección's biometrician, Br. V. Gonzalez-Panizza, for his great assistance in the evaluation of the data.

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Discussion

DR. NICHOLSON J. EASTMAN, Baltimore, Md.—Over the past 2 years approximately 160 taps of the full-term pregnant uterus have been made in our clinic for the purpose of direct study of the hemodynamics of the intervillous space. This work was done by Dr. Harry Prystowsky, Dr. André Hellegers, and Dr. Paul Bruns. As we all know, in about half of all pregnancies the placenta is located on the anterior surface of the uterus and in the other half on the posterior surface. In these 160 uterine taps we obtained blood from the intervillous space in approximately 80 cases and in the others we collected amniotic fluid. That it actually is intervillous space blood which is obtained in the successful uterine taps is supported by what seems to us uncontestable evidence of various kinds, but because of time limitations I will not go into that aspect of the studies. In the early days of this work these taps were done on the resting uterus, that is, between contractions. As chance would have it, before very long, when the investigator was inserting his needle, the patient started to have contractions, and he went ahead and inserted the needle into the intervillous space. After this had happened several times, it became apparent that the observations made in these taps in the presence of a contracting uterus were quite different from those made in a resting uterus. In the first place, whereas in a resting uterus it is possible to get only 4 or 5 c.c. of blood, one can get 8 to 10 c.c. when the uterus is contracting. Moreover, when the needle is put into the intervillous space in a resting uterus, one has to use negative pressure and withdraw the piston to get the blood out, whereas during a contraction only slight negative pressure is necessary to collect blood.

An effort was made to quantitate these findings. The pressure of the blood in the intervillous space of a resting uterus was found to be approximately 10 mg. Hg, but in the contracting uterus the pressure is increased almost fourfold. The amniotic fluid pressure in the resting uterus was found to be quite similar to the pressure in the intervillous space, but this relationship does not exist at the height of a contraction, when intervillous pressures are higher than intra-amniotic.

At the same time we were doing this work, Dr. Elizabeth Ramsay and Dr. George W. Corner, Jr., were carrying out identical experiments on pregnant monkeys near term. By chance, or more likely because of the manipulations of the uterus incident to the experiment, they had two monkeys in which labor started with the animals on the table with needles in the amniotic cavity and intervillous space, and they recorded the amniotic fluid pressure and blood pressure in the intervillous space. They found the same thing as we did in the human, namely, that the pressure in the intervillous space increases with each contraction to a pronounced degree and that the amniotic fluid pressure is always less than that in the intervillous space.

On the basis of these observations it is our feeling that the old idea that contraction of the uterus squeezes the intervillous space to such a degree that it is completely evacuated, is no longer tenable. Rather we now conceive of the intervillous space during contraction as being distended. Dr. Hendricks is an experienced and astute investigator, and in his

paper he gave considerable attention to this concept that I have laid before you in what he calls the trapping theory. It is his feeling that blood *is* squeezed out from the uterus into the systemic circulation. It might seem offhand that these two theories are conflicting but actually, as he pointed out at the close of his paper, there is no conflict between them and, as I see the situation, they fit together beautifully.

Dr. Hendricks, as I visualize what happens during uterine contraction, with the help of your findings it seems to me that at the beginning of a contraction the blood in the veins of the uterus is squeezed into the systemic circulation. That brings about an increase in the stroke output of the heart. As soon as that contraction reaches a peak, we know from these pressure readings that no more blood can get out of the uterus because the amniotic pressure is greater than the venous pressure. Then we are at the acme of contraction. You have shown that when you get near the acme of contraction the cardiac output begins to fall. Correlating that finding with what I have said about distention of the uterus, it seems to me that since the arterial pressure is always greater than amniotic pressure, we can conceive that at the acme of a contraction and with the venous flow shut off, the blood is pumped into the intervillous space which becomes distended. This should also explain the fall in cardiac output which you show beginning before or near the acme of a contraction. In other words, bringing these two concepts together, we have a running picture of a process that is very important and very vital to the fetal welfare.

DR. ARTHUR T. HERTIG, Boston, Mass.—I want to ask Dr. Hendricks whether it is possible, in relation to what Dr. Eastman has said, that with the onset of uterine contraction this enormous so-called marginal villous sinus would become compressed or in some way obstructed? Following such obstruction would the so-called true marginal sinus described by Spanner—the enormous accentuation of the intervillous space—become distended? Would this explain the concept that both you and Dr. Eastman have presented?

DR. HENDRICKS (Closing).—Dr. Eastman has pointed out that the process of extrusion cannot account for all the hemodynamic alterations which occur during uterine contraction, and with this I heartily agree.

Dr. E. J. Quilligan and I, studying human subjects in labor, have been doing some work similar to that reported by Dr. Eastman. We insert one catheter into the amniotic fluid space and another one into the intervillous space, carrying out simultaneous and continuous recordings over relatively extended periods of time. In the subjects studied there has been recorded a surprising amount of agreement between the pressures in the two spaces. In fact, from our work thus far it has appeared that the pressures in the amniotic fluid space and the intervillous space are virtually identical at any given instant of the contraction cycle. A report of this work is being prepared for publication.

In answer to Dr. Hertig's question, I do not know whether or not the marginal area of the maternal placental circulation receives any special "squeezing out." I would hazard the guess, however, that the entire intervillous space functions pretty much as a unit, perhaps having its volume mildly and uniformly compressed during the early phase of the contraction cycle and then sharing, again rather uniformly, an increasing distention which goes on after the contraction has proceeded far enough to bring about temporary occlusion of the uterine venous drainage. Such a concept appears reasonable in a system wherein the pressures both of the amniotic fluid and of the blood of the intervillous space were identical or nearly identical.

MEASUREMENTS OF PLACENTAL FUNCTION* †

E. Stewart Taylor, M.D., Paul D. Bruns, M.D.,
Harold J. Hepner, M.D., and Vera E. Drose, B.A.,
Denver, Colo.

(From the Department of Obstetrics and Gynecology, The University of Colorado Medical Center)

MANY clinical investigators are trying to gain understanding of the uteroplacental circulation as it relates to growth, development, and oxygenation of the fetus. Wright[1] has estimated effective uterine blood flow during labor using the radioactive sodium clearance method. His technique was to inject radioactive sodium into the myometrium during labor and measure its disappearance rate. Walker[2] has suggested that postmaturity may be complicated by chronic intrauterine fetal anoxia. Clifford[3, 4] has called the placental dysfunction syndrome to the attention of pediatricians. His clinical observations are similar to those made by Ballantyne[5] in 1902. Prolonged labor and toxemia of pregnancy are accompanied by uterine hypertonus which reduces effective uterine circulation.[6, 7] McKay[8] and Prystowsky[9] have each, by different methods, shown that toxemia of pregnancy interferes with oxygen transport from maternal to fetal blood. Assali,[10] by catheterization of the uterine vein, has calculated that the normal placenta clears 750 ml. of blood in one minute. Browne,[11] by radioactive sodium clearance methods, estimates placental blood flow at 600 ml. a minute in the normal subject near term. He also suggests that the normal placenta possesses approximately three times the amount of functional capacity required for normal pregnancy, labor, and delivery. Since many of the complications of pregnancy are accompanied by decreased placental function, it is desirable to know the degree of placental impairment before the onset of labor, or before term is reached in many instances.

This paper is a report of our experience with the measurement of estrogen and pregnanediol during normal and abnormal pregnancy and the measurement of radioactive sodium clearance from the myometrium during normal and abnormal pregnancy. An effort has been made to determine the value of these procedures as indicators of placental sufficiency before labor. One measurement of placental function is a quantitative measurement of the excreted products of placental metabolism. A second possible measurement of placental function is the uterine clearance time of radioactive sodium.

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We have asked the following questions in regard to the excretion of the metabolites of the placental hormones in the urine of the pregnant woman, and the significance of a prolonged clearance time of injected radioactive sodium.

1. What is the significance of a persistent low level of estrogen in the urine of a pregnant patient?

2. What is the significance of a prolonged uterine clearance time of radioactive sodium in a particular pregnancy?

3. May a patient with a low urinary estrogen have a normal pregnancy?

4. May a patient with prolonged uterine clearance of radioactive sodium have a normal pregnancy?

5. What is the significance of a low excretion of urinary pregnanediol? May a normal pregnancy result?

6. Are the urinary steroid tests and the uterine clearance test valuable as indices of placental function?

In this presentation the following assumptions are made: (1) that urinary estrogens and pregnanediol are of placental origin and therefore are a reflection of placental function, and (2) that the disappearance rate of radioactive sodium from the myometrium is an index of uterine circulation and therefore an indirect evidence of placental circulation.

Methods and Materials

A total of 57 pregnancies have been studied from the sixteenth gestational week with serial estrogen urine determinations. Each patient has had three or more separate determinations performed. Fifty-eight patients have had serial sodium pregnanediol determinations performed during pregnancy. The estrogen and pregnanediol excretion results have been studied in relation to the outcome of the particular pregnancy. The radioactive sodium clearance times obtained during pregnancy have been analyzed in relation to the results of the individual pregnancies. The estrogen determinations were performed by colorimetric assay, and the pregnanediol determinations were done by a gravimetric method. The details of both methods have been previously published.[12, 13]

One hundred thirty-nine patients have had uterine radioactive sodium clearance studies performed during the last 20 weeks of gestation. The details of this method have also been reported.[14, 15]

Results

1. Normal Estrogen Excretion (Table I).—Thirty-four patients studied were found to have normal urinary estrogen excretion. Our range for normal pregnancy has been published and corresponds with that of others.[16] The thirty-four normal determinations fell within the range shown in Fig. 1 or were higher.

Twenty-eight, or 82 per cent of the patients with normal estrogens, had a normal pregnancy and a normal full-term infant. Three patients with normal urinary estrogens were delivered prematurely. Three other patients had mild complications of pregnancy which in more severe forms may be associated with placental dysfunction. There was no evidence of placental insufficiency among these latter 3.

TABLE I. NORMAL ESTROGEN EXCRETION

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT*
Normal	28	82	
Pathological	6	18	
Two patients, premature delivery at 34 weeks			
Habitual prematurity			1,515 grams, neonatal death
Diabetic, surgical induction			2,250 grams, neonatal death
Diabetic, spontaneous delivery			
Multiple myomas			
Total patients studied	34		

*Normal full-term infant unless otherwise indicated.

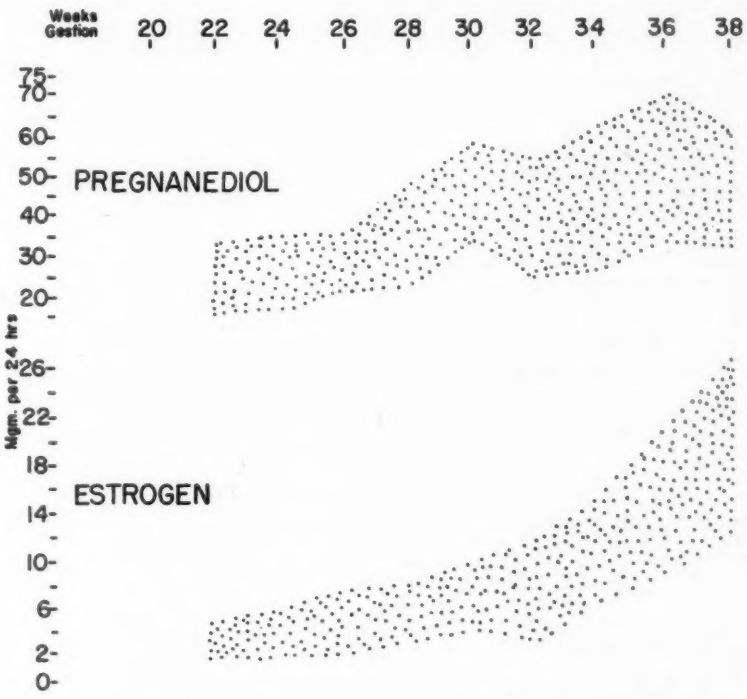

Fig. 1.—Normal values for estrogen and pregnanediol excretion in the urine from 34 patients who had normal pregnancies, labor, and deliveries.

2. Low Estrogen Excretion (Table II).—There were 23 patients in this group. Six, or 26 per cent, of our group who had an abnormally low or presumed inadequate production of estrogen from the placenta had entirely normal pregnancies, labors, and infants. A patient was considered to have low estrogens when more than half of the serial estrogen determinations were below the range of normal shown in Fig. 1. Seven, or 30 per cent, of the group had mild to severe pre-eclampsia. Fourteen per cent (3 patients) had premature delivery. Another 7 patients with low estrogens had a variety of conditions which led to poor placental development, and fetal or infant death resulted in 3 instances. The 17 patients in this group who had low estrogen values and possible inadequate placental function are represented in Fig. 2. Comparison of the pathological pregnancies in Table II with the pathological group in Table I reveals considerably more maternal and fetal disease in the pathological group with low estrogens.

TABLE II. LOW ESTROGEN EXCRETION

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT
Normal	6	26	
Pathological	17	74	
Three patients, premature delivery			2,200, 2,420, and 2,480 grams
Four patients, mild pre-eclampsia			
Severe pre-eclampsia; induction at 36 weeks			2,230 grams
Severe pre-eclampsia			
Severe pre-eclampsia			1,430 grams
Habitual premature labors			2,340 grams, neonatal death
Rh negative, sensitized			Erythroblastosis fetalis, 2,470 grams
Adenomyosis			2,400 grams
Adenomyosis			560 grams, stillborn
Multiple myomas with prolapsed uterus			1,500 grams, stillborn
Habital prematurity			2,180 grams
Chronic renal disease			
Total patients studied	23		

3. Normal Pregnanediol Excretion (Table III).—Serial pregnanediol determinations were performed on 34 patients during pregnancy who had normal pregnanediol excretion curves (Fig. 1). Nineteen (56 per cent) of these patients had normal pregnancies. Six (18 per cent) had mild to severe pre-eclampsia. Three more (9 per cent) had premature delivery. Another group of 6 patients had conditions which might be suspected to be associated with placental insufficiency.

TABLE III. NORMAL PREGNANEDIOL EXCRETION

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT
Normal	19	56	
Pathological	15	44	
Three patients, mild pre-eclampsia			
Moderate pre-eclampsia			1,868 grams
Severe pre-eclampsia			4,100 grams, Stillborn
Severe pre-eclampsia			2,220 grams
Three patients, premature delivery			2,130, 2,420, and 2,420 grams
Multiple myomas			
Diabetes, labor induced at 38 weeks			2,250 grams, neonatal death
Diabetes, spontaneous delivery			
Habitual prematurity			2,180 grams
Habitual prematurity			1,515 grams, neonatal death
Chronic renal disease			
Total patients studied	34		

4. Low Pregnanediol Excretion (Table IV).—Twenty-four patients with serial pregnanediol studies had low pregnanediol excretion patterns (more than half the determinations were below the standard deviation for normal). Eleven (46 per cent) of the patients in this group completed successful and normal pregnancies. The other 54 per cent had some complication of pregnancy accompanying this low pregnanediol excretion.

5. Normal Sodium Clearance Rate From Myometrium (Table V).—According to results of experiments in our laboratory, which correspond with those of other investigators, the radioactive sodium clearance time as expressed in half-time values is 1 to 6 minutes for normal pregnancy.[14, 15, 17, 18] Half-time value is defined as the length of time that is necessary for one-half of the injected radioactive sodium to disappear from the myometrium as measured by an end-window Geiger tube attached to a rate meter. This part of the report deals with the results of pregnancies of patients who had normal sodium clearance values from the myometrium according to this standard. Ninety patients had

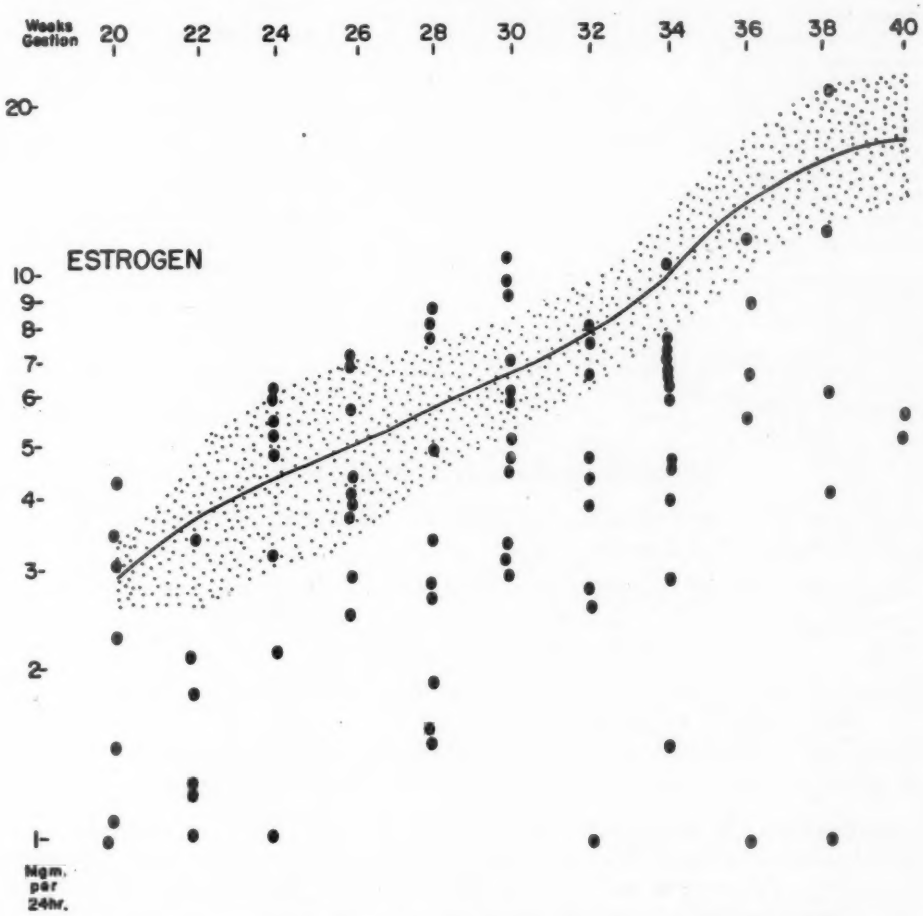

Fig. 2.—Seventeen patients with over half the serial estrogen determinations below the normal range. The solid line is the mean estrogen level for normal pregnancy. The shaded area represents one standard deviation from the mean. The 17 patients are listed in Table II as having possible uteroplacental dysfunction. As term is approached the estrogen excretion appears to be reduced in this group of patients rather than increased as expected.

clearance times that ranged from 1 minute to 5 minutes and 52 seconds. Sixty-six (73 per cent) of the normal clearance times occurred in patients who had normal pregnancies. Inspection of the 27 per cent of patients classified as pathological in Table V discloses that the majority of these patients had only mild to moderate degrees of obstetrical abnormality.

6. Prolonged Sodium Clearance Rate From Myometrium (Table VI).—A uterine clearance time of over 6 minutes was associated with complications of

pregnancy in 38 of 49 patients (77 per cent). Twenty-three per cent of prolonged uterine clearance times occurred in normal pregnancies. Several toxemia patients had delayed clearance times. Maternal infections, uterine bleeding, marked edema, and postmaturity appear to cause a prolonged myometrial clearance of radioactive sodium.

TABLE IV. LOW PREGNANEDIOL EXCRETION

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT
Normal	11	46	
Pathological	13	54	
Mild pre-eclampsia			2,300 grams
Two patients, mild pre-eclampsia			
Severe pre-eclampsia			
Severe pre-eclampsia, labor induced at 36 weeks			2,230 grams
Severe pre-eclampsia			1,430 grams
Two patients, premature delivery			2,340 and 2,480 grams
Rh negative, sensitized			Erythroblastosis fetalis, 2,470 grams
Adenomyosis			2,400 grams
Adenomyosis			560 grams, stillborn
Pneumonia			2,200 grams
Multiple myomas with prolapsed uterus			1,500 grams, stillborn
Total patients studied	24		

Statistical Analysis of Results

1. Formula.—The following formula was used for analyzing the results of tests for uteroplacental function:

Standard error of the difference between two proportions[19]:

$$\sqrt{(p_1 \times q_1)/n_1 + (p_2 \times q_2)/n_2}$$

p_1 and q_1 = the percentages in one sample which have and do not have the characteristic being discussed.

p_2 and q_2 = the corresponding percentages in the second sample.

n_1 and n_2 = the numbers of observations in the two samples.

Significance.—If two proportions differ by more than twice the value of the standard error of the difference, the difference is said to be significant at the 5 per cent level of confidence, and if they differ by more than three times the standard error of the difference, the difference is said to be significant at the 1 per cent level of confidence.

2. Estrogen Results.—Normal estrogens accompanied normal gestation in 82 per cent of 34 patients (S. E. ± 6.6 per cent). Low estrogens accompanied normal gestations in 26 per cent of 23 patients (S. E. ± 9.1 per cent). Since the difference in these percentages is four times as great as the standard error, these results would not occur by chance alone oftener than 6 times in 1,000 which is beyond the 1 per cent level of confidence.

Low estrogens accompanied pathological gestations in 74 per cent of 23 patients (S. E. ± 9.1 per cent). Normal estrogens accompanied pathological pregnancies in 18 per cent of 34 patients (S. E. ± 6.6 per cent). Again the difference of these percentages is four times as great as the standard error.

TABLE V. NORMAL UTERINE CLEARANCE TIME (1 MINUTE TO 5 MINUTES AND 52 SECONDS)

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT
Normal	66	73	
Pathological	24	27	
Two patients, mild pre-eclampsia			
Mild pre-eclampsia			2,200 grams
Mild pre-eclampsia, labor induced at 34 weeks			2,300 grams
Moderate pre-eclampsia			
Chronic hypertension, cesarean section at 35 weeks			2,400 grams
Chronic hypertension			
Nine patients, premature delivery			1,680 to 2,490 grams
Vaginal bleeding			
Septate uterus			2,120 grams
Three patients, diabetes			
Premature separation of placenta			1,990 grams
Rh negative, sensitized			Erythroblastosis fetalis, 2,470 grams
Habitual prematurity			2,340 grams, neonatal death
Total patients studied	90		

TABLE VI. PROLONGED UTERINE CLEARANCE TIME (LONGER THAN 6 MINUTES)

PREGNANCY, LABOR, AND PUERPERIUM	NO. OF PATIENTS	%	INFANT
Normal	11	23	
Pathological	38	77	
Six patients, mild pre-eclampsia			
Two patients, moderate pre-eclampsia			
Moderate pre-eclampsia			2,460 grams
Severe pre-eclampsia			2,150 grams, neonatal death
Unclassified toxemia			
Three patients, chronic hypertension			
Two patients, premature delivery			2,370 and 2,420 grams
Carcinoma of thyroid, operation			
Acute cholecystitis			
Vaginal bleeding			
Vaginal bleeding			2,200 grams
Abruptio placentae			1,920 grams
Ulcerative colitis			
Pyelitis			
Postural hypotension			
Three patients, marked edema			
Two patients, postmaturity			
Incompetent cervical os, operation			
Chronic renal disease			
Two patients, twins			1,770 and 2,090, 1,750 and 1,850 grams
Twins			
Multiple myomas			
Active syphilis			
Congenital abnormality of uterus			
Habitual prematurity delivery			1,910 grams
Total patients studied	49		

3. Pregnanediol Results.—Inspection of Tables III and IV shows at a glance that pregnanediol determinations are of no aid as indicators of uteroplacental function. There is no statistically significant difference when one compares the number of patients with normal and abnormal pregnancies with the pregnanediol excretion levels.

4. Uterine Clearance Time Results.—Seventy-three per cent of 90 patients had normal uterine clearance times and had normal pregnancies (S. E. ± 4.3 per cent). Prolonged uterine clearance times occurred in normal pregnancy in 23 per cent of 49 patients (S. E. ± 6.0 per cent). The difference in these percentages is five times as great as the standard error and these results would not occur by chance alone oftener than 5.73×10^{-5}. This same ratio is apparent when the pathological gestations are compared. Prolonged uterine clearance time characterized pathological pregnancy in 77 per cent of 49 patients (S. E. ± 6.0 per cent). In 27 per cent of 90 patients a normal uterine clearance time was accompanied by a pathological pregnancy (S. E. ± 4.3 per cent).

Comment

The methods presented in this report are imperfect for estimating placental reserve. Improved techniques which embody the principles of measuring placental metabolites or myometrial clearance tests may be of value in the solution of problems which require a knowledge of placental reserve. We recognize that there is a need for improved methods of estrogen determination. Brown's[20] method, introduced in 1955, has recently been adopted in our laboratory. The Brown method[21] seems to be more accurate than the methods previously reported. The methods used in the current report have given us consistent results on individual specimens of pregnancy urine and our patterns for the normal have been consistent. We are unable to explain why 26 per cent of patients with low levels of urinary estrogens had normal gestations. A normal urinary estrogen curve throughout pregnancy usually signifies normal uteroplacental function. A low level of estrogen cannot be interpreted as significant if the pregnancy is progressing normally. If a patient has one of the complications of pregnancy which is accompanied by placental insufficiency, we would attach some significance to a persistent low excretion of estrogens in the urine. Pregnanediol determinations in our hands do not promise to be of aid in the delineation of placental function. Estrogen determinations do hold some promise for the future; however, the effort and the expense involved in urinary estrogen determinations rules them impractical at this time for clinical practice. Zondek,[22] however, has used estrogen determinations during the last 20 weeks of pregnancy to measure placental function and has found them of significant value for this purpose.

The uterine clearance of radioactive sodium may be more practical than determinations of urine estrogens. Though subject to technical error this method does offer a potential aid in the estimation of placental efficiency. When applied to 2 postmature patients, the uterine clearance test was prolonged. One of the infants showed the effects of chronic anoxia at birth; the other did not. In our diabetic patients studied, the uteroplacental circulation

appeared normal according to this test. Edema, twin gestation, maternal infections, and toxemia all seem to interfere with uteroplacental function according to the sodium clearance test.

If a patient has repeated mid-trimester abortions or repeated premature delivery and normal uteroplacental function, one might suspect that early emptying of the uterus is secondary to cervical incompetence. We have two such patients who had normal placental function but had repeated mid-trimester abortions. Both of these patients had full-term pregnancies after surgical closure of the incompetent cervical os.

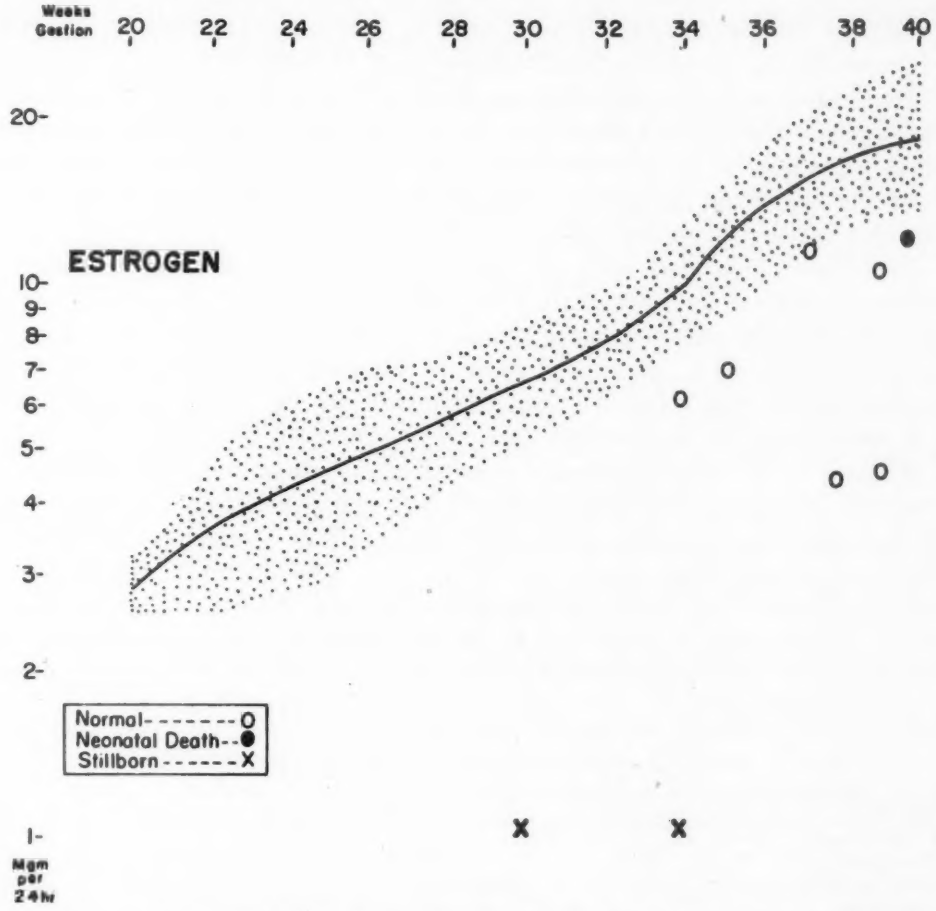

Fig. 3.—Nine patients with suspected placental dysfunction who had low estrogen excretion before labor.

We have noted that a normal estrogen excretion pattern and myometrial clearance time may change abruptly with an acute intercurrent infection such as pyelitis or pneumonia. The myometrium becomes irritable when a maternal infection is present and the patient may be delivered prematurely. Control of the maternal infection is accompanied by return to normal estrogen excretion and return to normal myometrial radioactive sodium clearance values.

Toxemia of pregnancy, depending on its degree of severity, has an effect on uteroplacental function. The more severe the toxic process the more this effect on myometrial clearance or estrogen excretion is noted.

Patients with twin pregnancies have normal estrogen-pregnanediol excretion. The uterine clearance time becomes abnormal with twin gestation, secondary to uterine distention.

Such conditions as multiple myomas of the uterus may have an effect on uterine clearance time and may explain why some infants fare badly in this environment.

Postural hypotension and excessive edema of pregnancy prolong the myometrial clearance of radioactive sodium. These mechanisms can be readily understood as reasons for temporary placental insufficiency.

General experience with kidney, liver, pulmonary, and cardiac function tests suggests that tests designed to estimate uteroplacental function may not always be as helpful as one might wish. Function tests are, nevertheless, some aid in the diagnosis, prognosis, and management of diseases of the kidney, liver, and cardiorespiratory systems.

We have performed both the urinary estrogen excretion tests and the radioactive sodium tests on 22 patients. In half this group both tests for placental function on the same patients gave parallel results for some degree of placental insufficiency. Since one type of test measures metabolic activity of the placental and the other is an indirect measurement of uteroplacental circulation, one would not expect them always to give parallel results.

Estrogen determinations made several days before delivery in 9 patients are shown in Fig. 3. These patients all had abnormally low estrogen values and had obstetrical complications that might affect uteroplacental function. There were 3 perinatal deaths in this group. The estrogen determinations were made before the fetal deaths. The degree of placental dysfunction in these 3 patients was presumed to be incompatible with infant survival. The other 6 infants survived pregnancy and labor. The patients and their infants are listed in Table VII.

TABLE VII. NINE PATIENTS WITH LOW ESTROGEN EXCRETION IN THE URINE*

COMPLICATION	INFANT
1. Pre-eclampsia	
2. Pre-eclampsia	Stillborn
3. Pre-eclampsia	
4. Nephritis	Neonatal death
5. Rheumatic heart disease	
6. Rheumatic heart disease	
7. Pneumonia	
8. Diabetes	
9. Purpuric disease	Stillborn

*Determinations were performed on 24 hour urine specimens, on 3 successive days while the patient was hospitalized for treatment of the pregnancy complication. The specimens were obtained between the thirtieth and fourtieth weeks of pregnancy.

Conclusions

1. A persistently low level of urinary estrogens was suggestive of uteroplacental dysfunction in 74 per cent of patients.

2. A prolonged uterine clearance time of radioactive sodium was indicative of uteroplacental dysfunction in 77 per cent of patients.

3. Twenty-six per cent of patients with low urinary estrogens during pregnancy had normal pregnancies.

4. Twenty-three per cent of patients with prolonged uterine clearance of radioactive sodium had normal pregnancies.

5. Pregnanediol determinations have not been of value to us in estimating placental function.

6. Estimation of total urinary estrogens, and determination of myometrial radioactive sodium clearance time may be of benefit in determining uteroplacental function in certain special circumstances.

We wish to acknowledge the aid of Dr. Edith Boyd of the Child Research Council at the University of Colorado Medical Center in the preparation of the statistical analysis of the data.

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Discussion

DR. LEROY A. CALKINS, Kansas City, Kan.—Dr. Taylor's objective is highly important and his data are statistically sound. If and when he compiles a considerably larger series, correlation of the various findings with placental weight at delivery would, I think, add materially to their significance.

We have long had the clinical impression that the prognosis for premature babies was considerably better if the placenta weighed 400 grams or more. Edith Potter's statement that "except with twins there is little evidence that fetal death is ever a result of insufficient placental size" makes one hesitate to speak very precisely.

Admittedly, placental weight is only one, and probably not the best, measure of placental sufficiency. Two other points had, however, come to our attention: (1) Premature separation of the normally implanted placenta rarely resulted in the loss of the baby if the placenta weighed 500 grams or more. (2) In our perinatal conferences, in almost all of the deaths for which "no cause" could be found the placentas weighed under 500 grams. Admittedly, many of these babies were so macerated as to defy proper postmortem examination but a considerable number could be thoroughly examined.

In 12,322 consecutive deliveries, gross developmental anomalies and abnormal presentations excluded, some very interesting findings were revealed (Table I).

TABLE I. PERINATAL MORTALITY PERCENTAGES

	PLACENTA UNDER 500 GRAMS	PLACENTA 500 GRAMS OR MORE
Premature separation	0.99	0.12
"No cause"	1.72	0.11
All other causes	1.78	0.56
	Term Pregnancies Only	
Premature separation	0.4	0.09
"No cause"	0.6	0.09
All other causes	0.4	0.56

It is quite obvious that, even after placentas up to 495 grams are included—usually considered quite an adequate weight—in the small-placenta group, striking differences appear between the two groups. Premature separation and "no cause" each produces a high proportion of the deaths in the small placenta group and a negligible proportion in the larger placentas. Combined they account for 71 per cent of the perinatal deaths in the former and only 25 per cent in the latter group.

It is our present impression that placental weights between 400 and 495 grams at full term are usually adequate unless there is premature separation, gross infarction, or some other impairment of function. While we have numerous examples of normal live birth at lesser weights, the mortality rate rises sharply with each step below 400 grams and is more often otherwise unexplained.

In this present series the mortality rate of babies between 1,500 and 1,995 grams was 29 per cent if the placenta weighed less than 400 grams, but only 16 per cent if it was larger.

DR. DANIEL G. MORTON, Los Angeles, Calif.—This question of the sufficiency of the placenta is certainly a most important one in any consideration of the causes of pregnancy wastage and of the birth of less than perfect babies. That the placenta usually starts out adequately endowed from the tissue point of view and remains so throughout pregnancy of normal term and even beyond, apparently with reserve to spare, is fortunate and accounts for the generally high incidence of the birth of normal, healthy babies. There is no living structure, however, which does not reproduce some defective specimens. And certainly not all living structures live to a ripe old age without suffering impairment of function or shortening of life because of adverse environmental conditions. The placenta is no exception. Placental insufficiency accounts for many abortions, some premature labors, some cases of "unexplained" premature death of the fetus in utero, the fetal malnutrition associated with the more severe grades of pregnancy toxemia, and the so-called postmaturity syndrome. The insufficiency can be severe enough to cause death of the fetus or mild enough to permit birth of a living but obviously undernourished child. It has been estimated by some that the normal healthy placenta has a reserve capacity three times that necessary for fetal good health and birth at term. If this is true, it is easy to understand why the postmaturity syndrome is encountered so frequently, even when pregnancy has gone 3 or 4 weeks past term. Inevitably, as time goes by, the aging process takes its toll and the margin between sufficiency and insufficiency becomes narrower, even as the margin between life and death narrows for all of us as each

day passes. There can be no doubt that in some instances, even in the absence of overt abnormalities, what with the addition of various factors, the placenta becomes significantly insufficient, resulting in the picture of impaired fetal nutrition, which Clifford has described so well, or in fetal death in utero.

The causes of placental insufficiency are not all known. We recognize (1) poor germ plasm, a hereditary factor; (2) natural aging, a process which all flesh is heir to but at different rates; (3) poor environmental conditions leading often to vascular and decidual changes unfavorable for the health of the chorion. Under this third heading one might include a great number of conditions such as hypertension, toxemia of pregnancy (cart or horse?), maternal infections, dietary factors, and so on. There may, of course, be other causes that we know not of, metabolic disturbances of obscure nature, and so on.

Certain manifestations of placental insufficiency, both anatomic and physiologic or metabolic, have been recognized, with the latter probably being consequences of the former. Hertig especially has studied this subject. He and McKay have described the degenerative changes of the trophoblast and cytotrophoblast and of the decidua which are associated with so many abortions. They have also described the deposition of fibrin in the intervillous spaces, leading to infarction and impairment of the blood supply of the villi, a frequent concomitant of toxemia of pregnancy. Of great interest too is their description of the thickening of the basement membrane of the villous capillaries seen in association with the postmaturity syndrome and in toxemia. From a functional standpoint these changes no doubt interfere with the two most important activities of the placenta that we recognize: the hormone-secreting and the transport mechanisms. Both appear to be essential for the prosperity of the pregnancy but whether deficiency in secretory ability can be correlated with deficiency in transport efficiency is questionable. We know already that the correlation is not perfect but it has not been clear heretofore to what extent one can apply observations regarding the efficiency of one of these functions to the other. Dr. Taylor's observations help to throw some light on this important subject.

In the past we have been hampered by not having sufficiently precise methods of hormone determinations and by not having sensitive enough measures of transport ability to be confident of the reliability of the data obtained.

In this dilemma we like the kind of attack Dr. Taylor and his associates have made upon the problem. They have employed hormone assay methods in which they feel reasonable confidence. Added to this they have used an indirect method of determining over-all placental well-being, the sodium clearance rate from the myometrium. The high correlation of estrogen deficiency with various clinical manifestations suggestive of placental deficiency is exciting. These findings are consonant with those of Zondek. We would like to see a greater variety of tests applied in individual cases, despite what we realize to be great practical difficulties. Dr. Taylor's 22 cases in which two tests were used do not give us a feeling of satisfaction that this point has been covered thoroughly enough.

DR. ARTHUR T. HERTIG, Boston, Mass.—Were any routine pathologic examinations made of the placentas of these babies? Dr. Calkins asked if any weights were recorded and I am curious as to whether this functional study could be correlated with old-fashioned pathologic anatomy.

DR. ALLAN C. BARNES, Cleveland, Ohio.—We must recognize fundamentally that we will have to approach the diagnosis of placental adequacy by indirection. So far the indirection revolves around the placenta, and Dr. Taylor's premise is that the observed excretion rates could be correlated with what the placenta could pass across to the baby. There are other end points that might be adopted that would revolve around the baby itself. If the baby is losing weight then there have to be some breakdown products of tissue destruction that the mother in turn is required to eliminate. Maternal urinary excretion studies of tissue breakdown products might give us a clue. We have been employing this particular approach only to run down blind alleys. Mucoprotein output on the part of the mother does not statistically bear out which babies are losing weight. Nucleoprotein excretion rates of the mother should go up if the baby loses weight but we

have exhausted that at the moment. There has to be somewhere something that the mother must eliminate during the time the baby is in distress. Any studies in this direction are tremendously valuable, or we will always be in the position of discovering in retrospect that a particular placenta was inadequate, without forewarning that there might be difficulty ahead.

DR. LOUIS M. HELLMAN, Brooklyn, N. Y.—It would be nice if we could achieve a test for placental efficiency before birth as good, for instance, as the renal clearance test. I would like to report one attempt which we have made in this direction. On several occasions we have injected two radioactive isotopes, namely, sodium and iodinated albumin, into the intervillous space. Theoretically, one of these substances, the albumin, will not cross the placenta. Therefore, the sodium, having two means of egress (the baby and the maternal circulation), should have a more rapid disappearance curve.

I regret to report to you that in the very few experiments we have done, the results turned out to be just the opposite: the sodium disappeared at a slower rate than the albumin. The reason for this is unknown.

DR. EDWARD C. HUGHES, Syracuse, N. Y.—I think we all agree that we are leaning toward an endocrine-steroid-electrolyte field in our specialty and that many of our problems will be decided at this level. For some time we have been interested in placental function and have attempted to find some satisfactory method which we could use to measure the output of substances necessary to sustain fetal life. We have followed normal patients throughout the entire gestation period, estimating the quantitative output of chorionic gonadotrophin and pregnandiol and other derivatives of this same nucleus. We find in normal patients that these relationships are constant: the output of gonadotrophin remains relatively low and the output of pregnandiol increases toward the end of gestation. We had 25 patients who gave bad obstetrical histories where these patterns were not maintained. In the first place, the gonadothropin did not rise high enough at the beginning of gestation and did not remain at normally low levels during pregnancy, and the pregnandiol output was below normal at this time. This occurred in diabetes and in 5 cases of brain anomalies, 2 of which patients have lived to become cerebral spastics. All placentas were weighed and studied chemically and histochemically. We will have a report on our findings later.

I think we must study placental function in all its phases because I am sure that the future development and growth depend upon the intricate mechanism involved in the metabolism of essential materials that establishes good nutrition for the embryo and fetus while in utero.

DR. THADDEUS L. MONTGOMERY, Philadelphia, Pa.—Up to the moment most studies of fetal distress have taken the form of an electrocardiography or electroencephalography has revealed some changes in fetal heart rhythm in response to hypoxia, yet the legible complexes have been difficult to secure and oftentimes the complexes show changes only when hypoxia and permanent damage to the fetus are well advanced. There is hope, however, that further information on the problem of hypoxia in utero may be obtained.

Our own experience with fetal electroencephalography has not been such as to indicate any significant alteration in the early stages of hypoxia. As a matter of fact, Dr. Richard Bernstine, working in our own department, has noted a decided tendency of the electroencephalographic waves to persist even after apparent somatic death. For instance, in a small nonviable late abortion, electroencephalographic waves were found to persist 25 to 30 minutes after arrest of heart action.

It is hoped that the work which Dr. Taylor is following may be useful in evaluating the progress of the pregnancy in complications such as toxemia of pregnancy and placental insufficiency. In the meantime, it is important to remember that a fairly accurate appraisal of the fetal condition can be made by accurate and prolonged auscultation of fetal heart sounds, particularly the careful observation of fetal heart sounds during and after contraction of the uterus. Occasionally the reaction of the fetal heart sounds to small-dosage oxytocin is also helpful.

Some years ago we instituted a "fetal heart survey" in all new admissions in labor and in all patients who were under treatment for toxemia of pregnancy. The survey consisted simply of an accurately charted record of the rate of fetal heart sounds as observed over a period of 20 to 30 minutes, particularly during and after each uterine contraction. By this means it has been possible to pick up instances of fetal distress in utero which would not be observed in episodic or occasional listening to heart sounds.

This method has proved useful also in instances where induction of labor has been contemplated in toxemia of pregnancy and where placental insufficiency is likely to be present. In such instances small-dosage oxytocin (¼ to ½ minim of the standard ampules) is employed to stimulate uterine contractions and a notation carefully made of the effect on the fetal heart.

While we are awaiting more scientific methods of evaluating the fetus in utero these time-honored clinical methods carefully pursued can still give quite useful data.

DR. TAYLOR (Closing).—In reply to Dr. Calkins, I must state that we have not correlated these data with the weight of the placenta. We have some histological data on the placentas but not enough to correlate. We will continue these histological studies.

We are holding the concept that the hormone secretion by the placenta reflects its vitality. The circulation in the myometrium may reflect the amount of blood available to the placenta for use in metabolism. We cannot expect these two tests to correlate exactly in each instance because different factors may interfere in either the placenta or myometrium.

From a practical point of view, these tests may be useful in the management of the diabetic patient at term, the patient with chronic hypertension, and possibly the syndrome of postmaturity. For instance, we now tend to deliver diabetic patients before term according to empiric rules. This is not right, because pregnancy should be interrupted before term in some patients, and others should continue to term, and yet no one knows really in which diabetic patient pregnancy should be terminated. Hypertensive patients with albuminuria or patients with postmaturity often pose a similar problem. I feel sure there are infants who are adversely affected by postmaturity, and I am sure that there are infants who are not, but I am uncertain how to tell the difference before labor.

STUDIES IN FETAL WELL-BEING: VARIATIONS IN FETAL HEART RATE*†

LOUIS M. HELLMAN, M.D., MORTON A. SCHIFFER, M.D., SCHUYLER G. KOHL, M.D., AND WALTER E. TOLLES, M.S., BROOKLYN, N. Y.

(From the State University of New York Downstate Medical Center and The Kings County Hospital, Brooklyn, New York, and Airborne Instruments Laboratories, Mineola, New York)

COULD the obstetrician but know the state of well-being of the infant in utero some decisions as to time and method of delivery, now almost intuitively made, might be determined on a more scientific basis. Particularly in instances of diabetes and toxemia an accurate assessment of fetal intrauterine health could be used as a gauge against which the perils of extrauterine prematurity could be nicely balanced. In a similar fashion such information would be of the utmost value during labor and delivery. While attempts have been made to achieve knowledge of fetal health during labor through correlation and evaluation of the obvious signs of fetal distress,[1-9] there are no available data which shed light on the status of the fetus prior to the onset of labor.

Recently some information has been gained as to the efficiency of the uterine and placental "circulation" and concerning some factors affecting the transport of oxygen.[10-13] It is as yet too early to assess these findings in terms of fetal well-being. Even if placental efficiency could be measured with the exactness of renal function, however, only one parameter of the fetal status in utero would be achieved. Unfortunately the human fetus is so inaccessible to study that only its heartbeat, infrequent movements, and the passage of meconium are measurable. Even in the adult, with many of his systems readily accessible to measurement, physiological well-being is but inexactly estimated. Small wonder then that the well-protected fetus, even in experimental animals, has defied such evaluation prior to labor and has, during delivery, yielded only the grossest indications of jeopardy.

One parameter that can be assessed prior to labor is the normal variation of fetal heart rate and rhythm. First heard by Mayor[14] of Geneva in 1818, the sound of the fetal heart was accidentally rediscovered by Lejumeau, Viscount of Kegaradec,[15] in 1821, while he was attempting to hear the splash of

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the fetus in its amniotic fluid with Lannaec's new stethoscope. So assiduously did he pursue his discovery and so meticulously did he record his observations that subsequent investigations have revealed little new. There is a vast literature on the significance of changes in the fetal heart rate associated with impending asphyxia. The fast beat, the slow and the irregular beat, and particularly the concomitant passage of meconium have been repeatedly studied. Although it is almost universally agreed that radical changes in rate, particularly if associated with the passage of meconium, are frequently signs of infant jeopardy the long range prognostic significance of any one or of combinations of these signs is by no means clear. The more sophisticated tools of phono- and electrocardiography have also been applied to the fetal heart in labor[16, 17, 18] and although some investigators claim wave changes in the electrocardiograph signature associated with hypoxia, these have not been confirmed. More recently rate meters have been devised for these instruments[19, 20] but to date increasing complexity of apparatus has proved costly without any substantial yield of new information.

The studies about to be reported are twofold in nature. First, an assessment of minute variations in the fetal heart rate and rhythm prior to labor and, second, an evaluation of the prognostic significance of the classic signs of fetal distress during parturition.

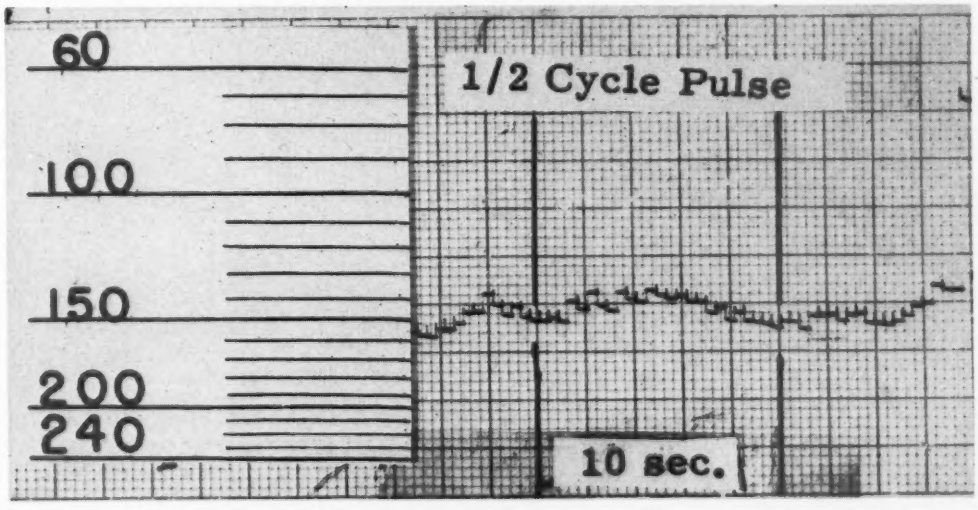

Fig. 1.—Typical slow variation in rate of fetal heart—10 second ½ cycle pulse of 13 beats per minute amplitude.

A thorough review of what had been achieved in recording and analysis of fetal heart tracings indicated the improbability of obtaining significant changes of signature from the fetal electrocardiograph, especially with the mother not in labor. Furthermore, while such an instrument might be built to record rate alone, there are difficulties in attaining sufficient sensitivity to record real wave forms and yet eliminate the maternal signature. On the other hand if one were content with rate changes alone a relatively simple phonocardiograph with an instantaneous rate meter might disclose hitherto unobserved minute variations in rate and rhythm which constitute a pattern of

normality. The analysis of such patterns and the discoverable factors that modify them might very well furnish a clue to the intrauterine health of the fetus.

A phonocardiograph with an instantaneous rate meter has been constructed which records the beat-to-beat frequency of the fetal heart. The design of such a machine presents certain complex problems, some of which are not yet solved in adult phonocardiography.[21] When one adds to these the inaccessibility of the fetus, with the many interfering sounds generated by the mother's body and the extraneous noises which her body picks up and amplifies, it is a small wonder that the machine records as well as it does.

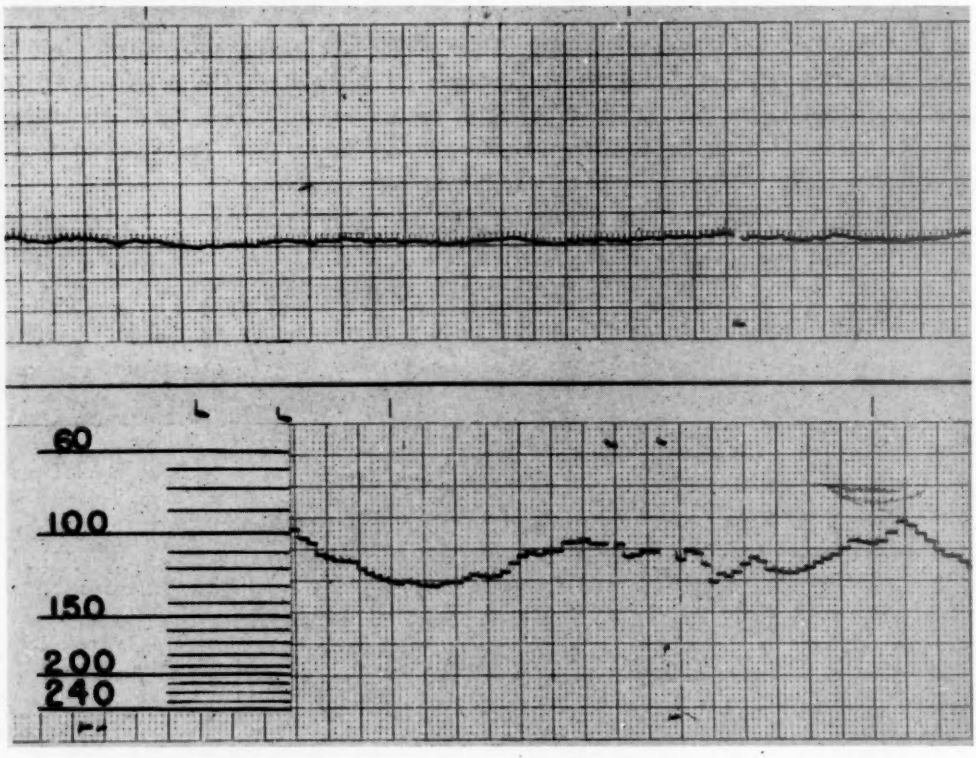

Fig. 2.—Fetal heart rate showing change from very small ½ cycle pulse to large. Cause unknown.

At the present stage of development, satisfactory records are obtainable in nearly 50 per cent of patients. Long-time tracings to show diurnal variations in frequency and rhythm are most difficult to obtain and the successful recording of the effects of stimuli tries the patience of the investigator. Undoubtedly the machine can and will be improved. Nevertheless, about 100 satisfactory records from both normal and abnormal pregnancies have been obtained and the preliminary presentation and analysis of these constitute the first part of this report.

The records are on standard electrocardiograph paper moving at the rate of 2.5 mm. per second. The rate is recorded on the vertical logarithmic scale. Variations in frequency take the form of mono- or diphasic waves or pulses. For the purpose of analysis these are measured as to the time and amplitude

of the monophasic wave ½ cycle pulse (Fig. 1). The small rectangular irregularities in the tracings are artifacts probably due to the complexity of the signature of the sounds.

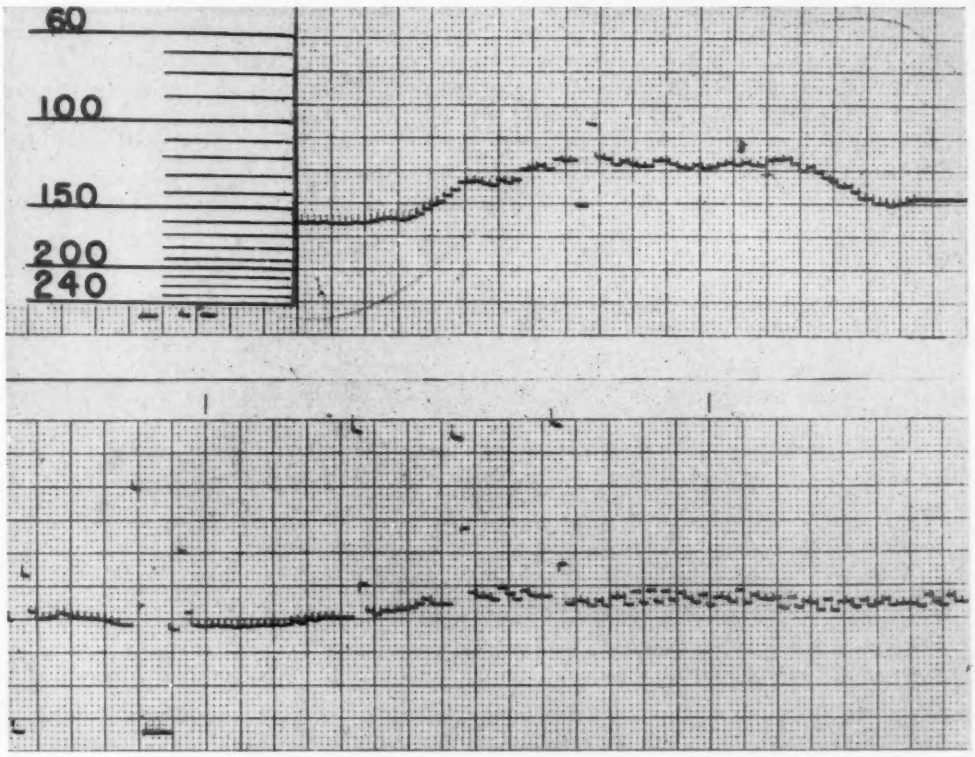

Fig. 3.—Change from large ½ cycle pulse to small. Cause unknown.

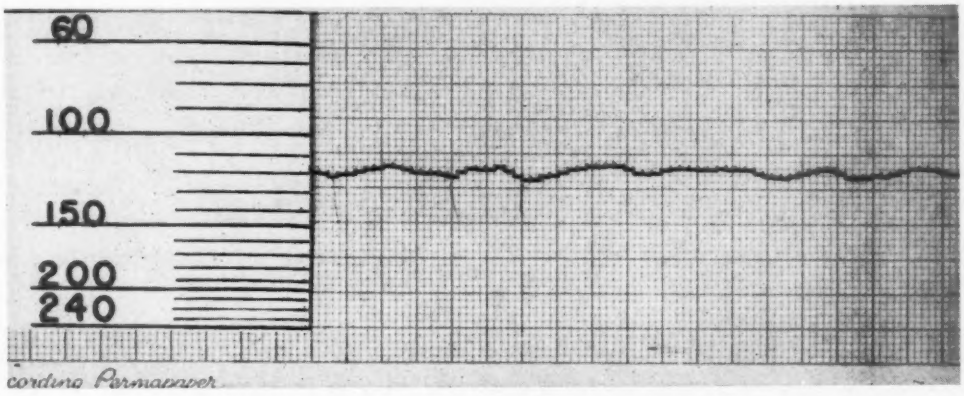

Fig. 4.—Fetal heart rate showing fairly regular phasic arrhythmia.

As with any biological rhythm the rate of the fetal heart could not be expected to be constant. Even the most steady tracing shows long slow waves of amplitude from 3 to 5 beats per minute. These are not characteristic of any one fetus for in a short space of time and without discernible cause the

pattern can change and become amplified to a point where the ½ cycle pulses vary as much as 30 beats per minute (Figs. 2 and 3). In other equally normal situations a fairly regular type of phasic arrhythmia prevails (Fig. 4).

Figs. 5 and 6 show tracings from twins, the first presenting by vertex and the second by the breech. The rates are only slightly different but the first twin shows quite a different phasic arrhythmia from the second. Furthermore, the signatures which are shown on these tracings are different. The signatures are taken in close association with but not at the same time as the rate tracings, and on paper moving 10 times faster than the latter. The difference in signatures could be intrinsic but it could equally well stem from differences in position of the twins and the amount of maternal tissue interposed between the heart and the microphone. The signature of the first heart sound of the first twin is much more complex than that of the second, however.

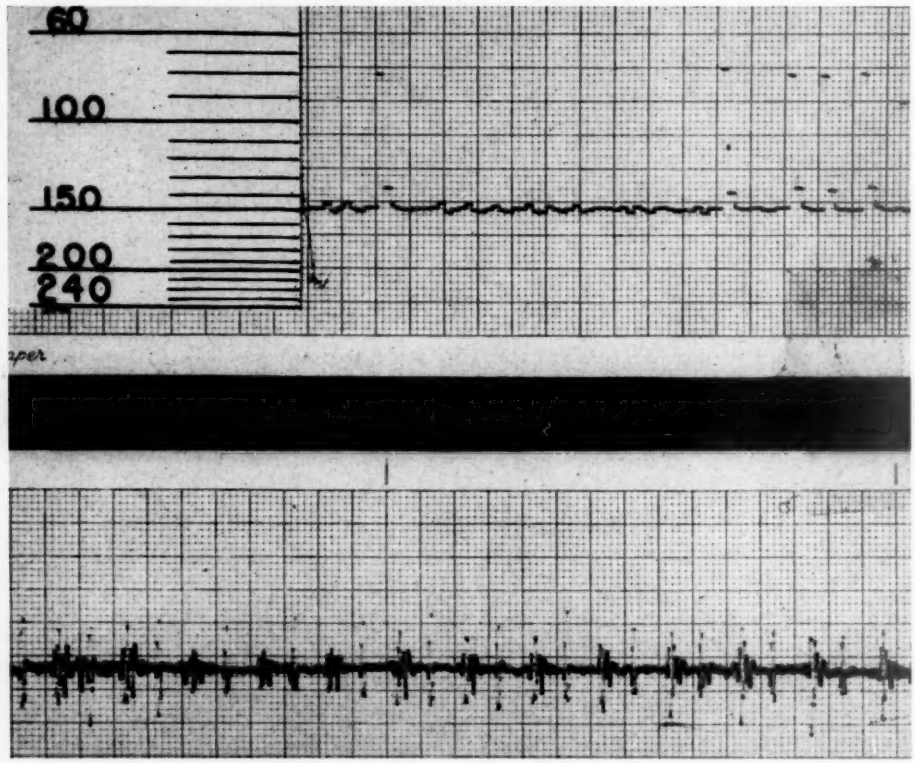

Fig. 5.—Fetal heart rate of first twin showing very small ½ cycle pulse and a complex signature of the first sound.

It is interesting to compare the fetal tracings with those of the newborn. As might be expected, the infant mirrors the fetus but with an increased intensity. The same irregular phasic arrhythmia is present with an exaggeration of the height and frequency of the ½ cycle pulses (Fig. 7). Superimposed on some are the beginnings of the respiratory sinus arrhythmia absent in the fetal heart (Fig. 8).

The maternal heart rate tracings show the same two types of arrhythmia, namely, that associated with respiration and the long slow ½ cycle pulses (Figs. 9 and 10).

Only a beginning has been made in the analysis of these data. In general, the ½ cycle pulses of the fetus increase with their duration (Fig. 11). The amplitude of the tracings of the mother and the newborn infant have not been analyzed in this fashion and inspection suggests that quite the opposite is true. On the other hand, histograms of the ½ cycle pulse frequency show a surprising similarity between the adult and fetal groups (Figs. 12 and 13).

In order to evaluate the long-accepted signs of fetal distress in labor, rapid, irregular, and slow fetal heartbeats and the passage of meconium have been recorded on our punch cards since 1951. A slow fetal heart rate is defined as a rate below 100 beats per minute while rapid is a rate above 180. Irregularity constitutes fluctations between these limits—all observations being made between contractions. The amount of meconium was not recorded.

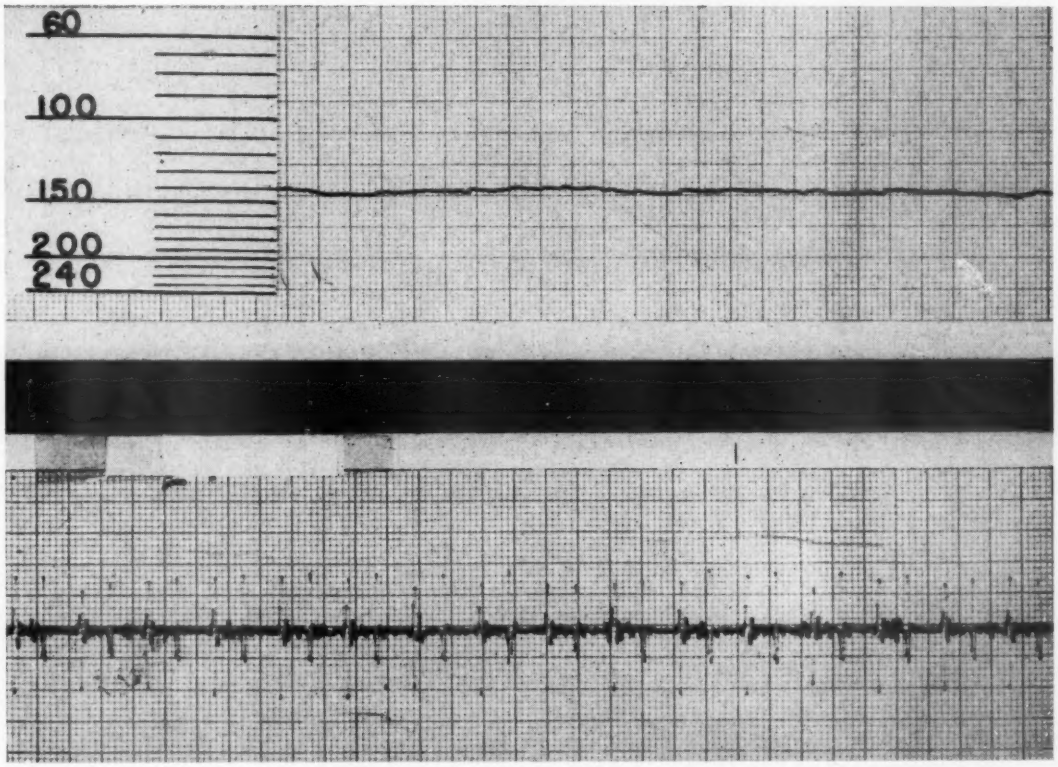

Fig. 6.—Fetal heart rate of second twin showing long ½ cycle pulse and fairly simple signature.

No special emphasis was put on the collection of these data although the labor records in general are quite detailed. Only single, term infants in vertex presentation and alive at the onset of labor are included in this study.

TABLE I. INCIDENCE OF FETAL DISTRESS IN LABOR,* KINGS COUNTY HOSPITAL, 1951-1956

DELIVERIES	FETAL DISTRESS	FREQUENCY
20,481	600	2.9

*Term infants alive at the onset of labor. Breech and twins excluded.

TABLE II. PERINATAL LOSS ASSOCIATED WITH VARIOUS SIGNS OF FETAL DISTRESS IN LABOR*

SIGN	NO.	LOSS	%
Meconium	341	24	7.0
Meconium + slow F.H.	25	5	20.0
Meconium + irregular F.H.	8	1	12.5
Meconium + slow and irregular F.H.	21	3	14.3
Slow F.H.	94	5	5.3
Slow and irregular F.H.	64	6	9.4
Irregular F.H.	32	2	6.3
Rapid F.H.	15	0	0.0
Total	600	46	7.7

*Term infants alive at the onset of labor only. Breech and twins excluded.

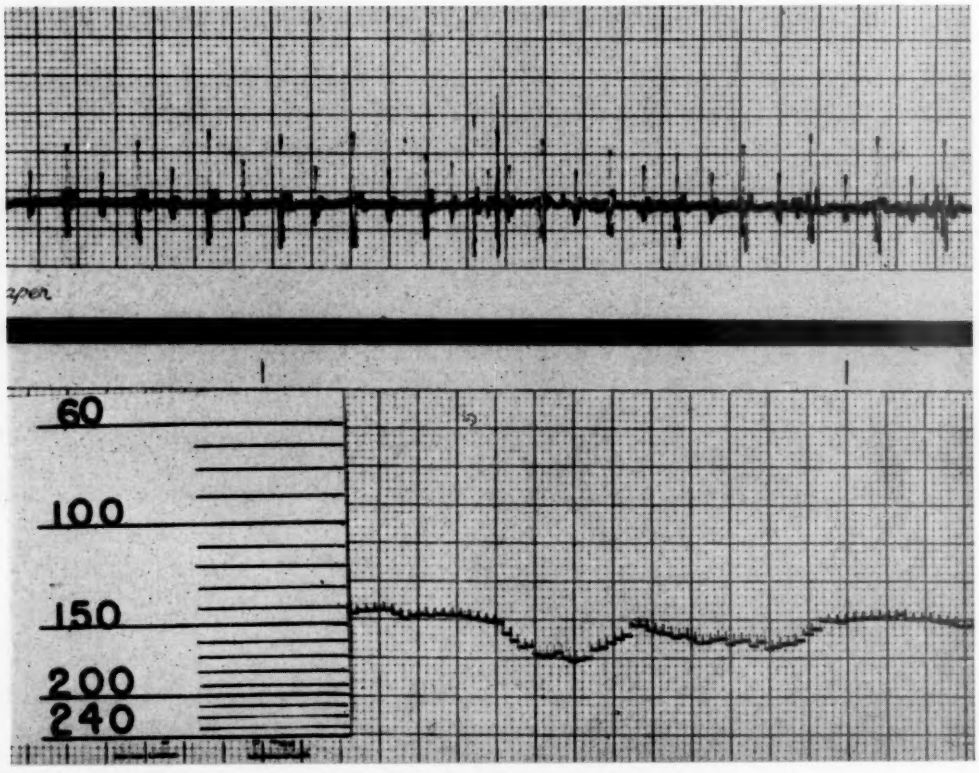

Fig. 7.—Heart rate of newborn infant showing irregular phasic arrhythmia typical of intrauterine existence.

TABLE III. PERINATAL LOSS ASSOCIATED WITH ALL SIGNS OF FETAL DISTRESS IN LABOR LISTED BY COMPLICATIONS*

COMPLICATION	NO.	LOSS	%
Uterine dysfunction	132	11	8.3
Postmaturity	63	3	4.8
Antepartum hemorrhage	29	3	10.3
Toxemia	47	3	6.3
Prolapsed cord	27	7	26.0
Abnormal presentation	6	0	0.0
None	296	19	6.4
Total	600	46	7.7

*Term infants alive at the onset of labor only. Breech and twins excluded.

TABLE IV. PERINATAL LOSS ASSOCIATED WITH SLOW FETAL HEART IN LABOR LISTED BY COMPLICATIONS*

COMPLICATION	NO.	LOSS	%
Uterine Dysfunction	23	0	0.0
Postmaturity	8	0	0.0
Antepartum hemorrhage	6	1	16.7
Toxemia	7	0	0.0
Prolapsed cord	9	3	33.3
Abnormal presentation	1	0	0.0
None	40	1	2.5
Total	94	5	5.3

*Term infants alive at the onset of labor only. Breech and twins excluded.

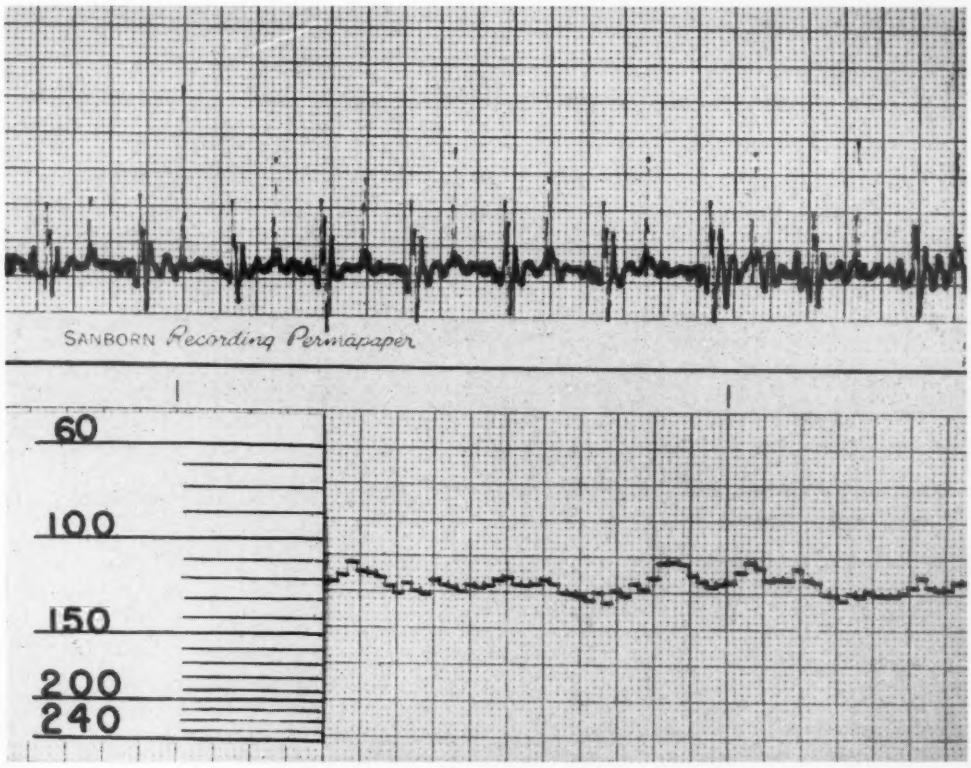

Fig. 8.—Newborn infant showing both irregular phasic arrhythmia and sinus arrhythmia associated with respiration.

There were 600 instances of fetal distress in 20,481 deliveries of such infants from 1951 to 1956, a rate of 2.9 per cent (Table I). The division into various signs and combinations of signs of distress with the associated perinatal loss is shown in Table II. It is surprising that over 50 per cent have the single sign—meconium. This in combination with heart rate abnormalities points to the gravest of all hazards to the fetus. So long as these data remain unpurified, however, it is difficult to assess their prognostic value as far as infant survival is concerned, for the cures include obstetrical difficulties which in themselves not only contribute to perinatal loss, but also indicate special methods of delivery. Table III shows the infant loss rates where signs of fetal distress are present, listed by the various complications. These rates are of the same order of magnitude for the clinic at large with these

specific complications. In other words, the addition of signs of fetal distress to an already major obstetrical difficulty does not appear to augment the infant loss rate.

This leaves the real point of interest in the infant survival in the cases which show specific signs of fetal distress but are free of major obstetrical complications. These are shown for single signs of fetal distress in Tables IV, V, and VI, while Table VII includes the rates for the various combinations of signs. It becomes quite apparent from these tables that, when specific obstetrical difficulties are removed, the passage of meconium carries by far the gravest import for fetal survival. Table VIII shows a regrouping of these data so that

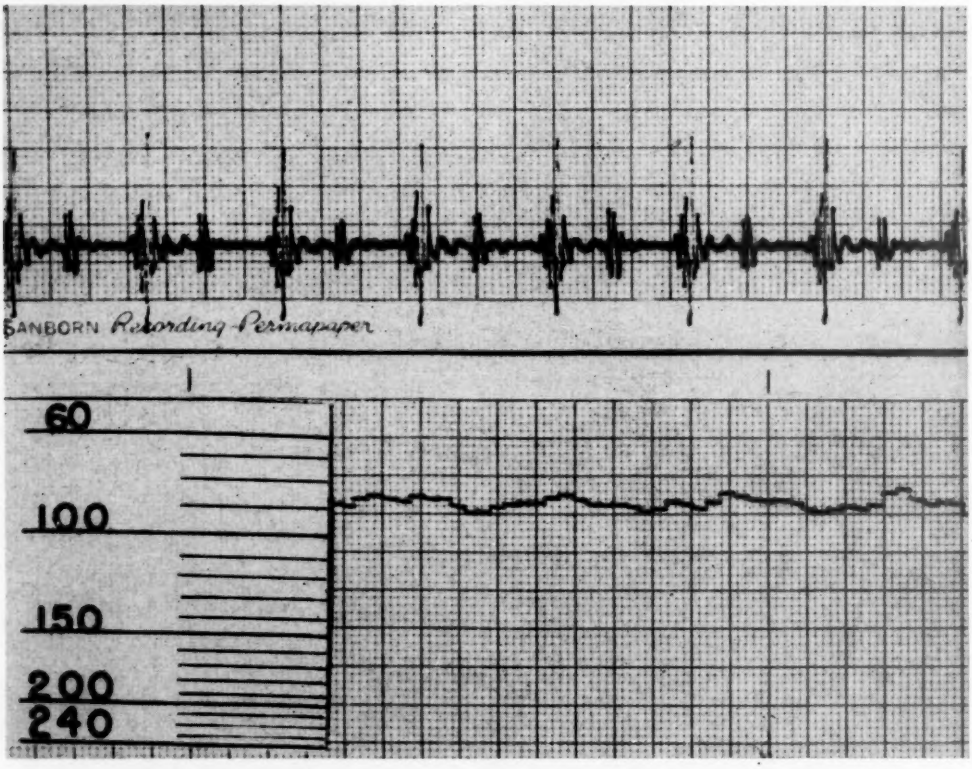

Fig. 9.—Maternal heart rate showing phasic arrhythmia not associated with respiration.

the loss rate associated with the passage of meconium alone and in combination with fetal heart abnormalities may be compared with the loss rate with fetal heart abnormalities alone. These groups are also listed with and without obstetrical complications. It is unfortunate that even with this very rich material the frequency of the signs of fetal distress is so small that the purification of the data to the point where they become clinically meaningful yields numbers too small for statistical validity. Table VIII, however, gives an indication of the serious prognostic significance of meconium whether it be associated with a major obstetrical complication or not. The basic infant loss rate in our clinic is 7 per 1,000 deliveries.* In the absence of obstetrical complication, the appearance of the classic signs of fetal distress are

*Perinatal loss rate for single, term infants alive at the onset of labor in deliveries with no obstetrical complications.

TABLE V. PERINATAL LOSS ASSOCIATED WITH IRREGULAR FETAL HEART IN LABOR LISTED BY COMPLICATIONS*

COMPLICATION	NO.	LOSS	%
Uterine dysfunction	8	2	25.0
Postmaturity	4	0	0.0
Antepartum hemorrhage	3	0	0.0
Toxemia	3	0	0.0
Prolapsed cord	3	0	0.0
Abnormal presentation	1	0	0.0
None	10	0	0.0
Total	32	2	6.3

*Term infants alive at the onset of labor only. Breech and twins excluded.

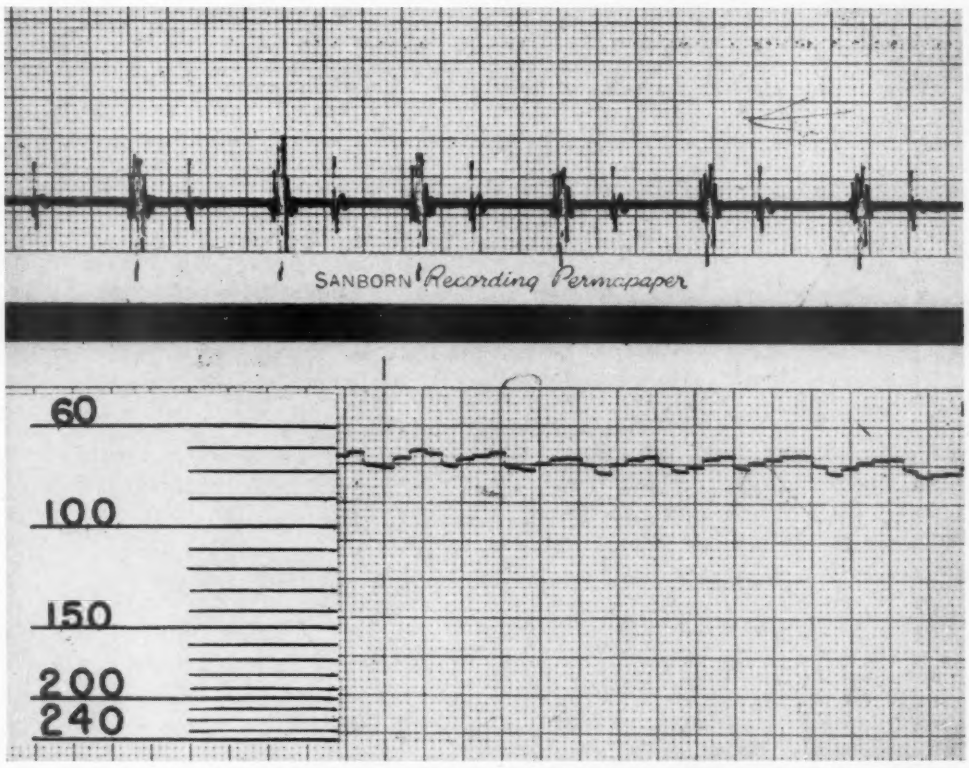

Fig. 10.—Maternal heart rate showing sinus arrhythmia associated with respiration.

TABLE VI. PERINATAL LOSS ASSOCIATED WITH MECONIUM IN LABOR LISTED BY COMPLICATIONS*

COMPLICATION	NO.	LOSS	%
Uterine dysfunction	62	5	8.1
Postmaturity	42	1	2.4
Antepartum hemorrhage	14	1	7.1
Toxemia	28	2	7.1
Prolapsed cord	5	0	0.0
Abnormal presentation	3	0	0.0
None	187	15	8.0
Total	341	24	7.0

*Term infants alive at the onset of labor only. Breech and twins excluded.

associated with a loss rate of 64. With meconium alone the fetal loss is over 11 times the basic rate and slightly over 3 times that associated with abnormalities of the fetal heart.

TABLE VII. PERINATAL LOSS ASSOCIATED WITH SIGNS OF FETAL DISTRESS WITHOUT COMPLICATIONS*

SIGN	NO.	LOSS	%
Meconium	187	15	8.0
Meconium + slow F.H.	14	1	7.2
Meconium + irregular F.H.	3	1	33.3
Meconium + slow and irregular F.H.	10	0	0.0
Slow F.H.	40	1	2.5
Slow and irregular F.H.	26	1	4.3
Irregular F.H.	10	0	0.0
Rapid F.H.	6	0	0.0
Total	296	19	6.4

*Term infants alive at the onset of labor only. Breech and twins excluded.

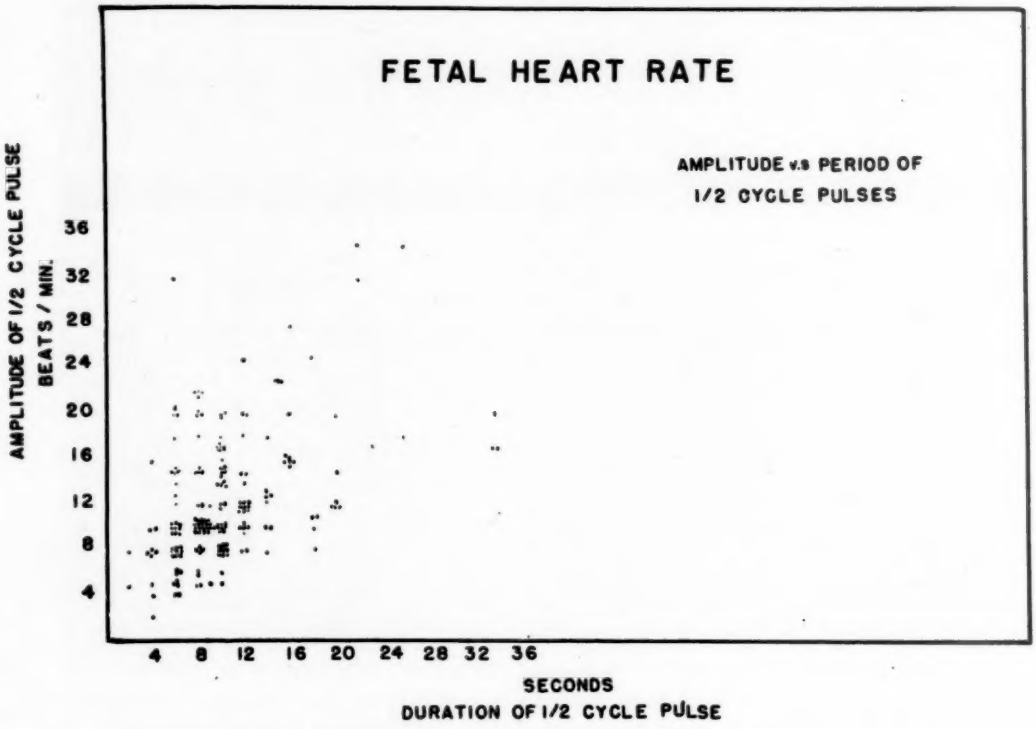

Fig. 11.

TABLE VIII. PERINATAL LOSS ASSOCIATED WITH SIGNS OF FETAL DISTRESS IN LABOR WITH OR WITHOUT COMPLICATIONS*

	NO COMPLICATIONS			COMPLICATIONS		
	NO.	LOSS	%	No.	Loss	%
Meconium	187	15	8.0	154	9	5.8
Meconium plus fetal heart abnormality	27	2	7.4	27	7	26.0
Fetal heart abnormality	82	2	2.4	123	11	8.9
Total	296	19	6.4	304	27	8.9

*Term infants alive at the onset of labor only. Breech and twins excluded.

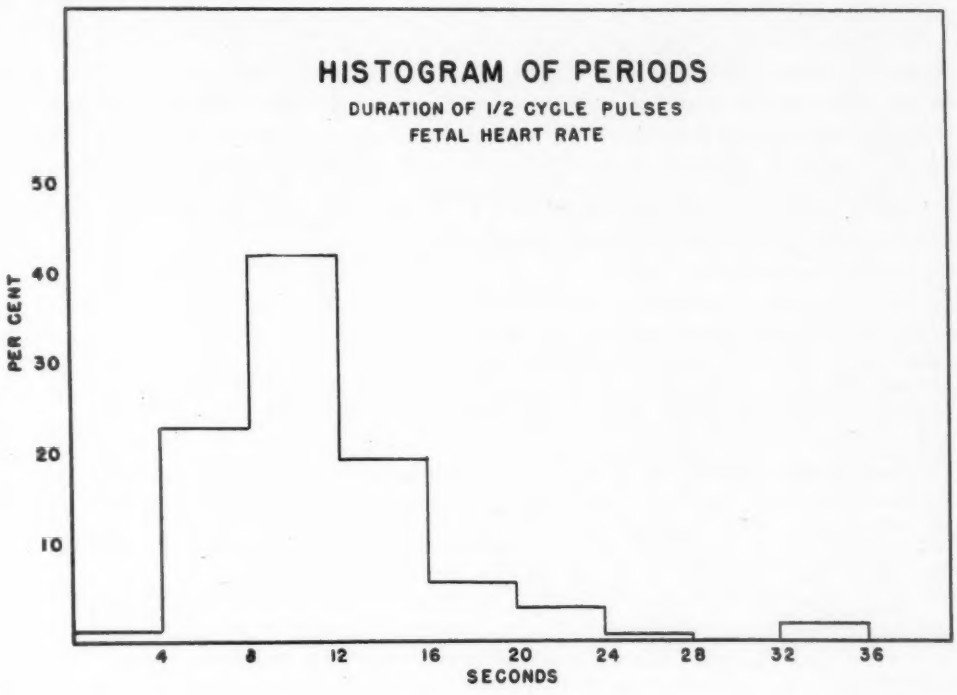
HISTOGRAM OF PERIODS
DURATION OF 1/2 CYCLE PULSES
FETAL HEART RATE
PER CENT
50
40
30
20
10
4
8
12
16
20
24
28
32
36
SECONDS

Fig. 12.

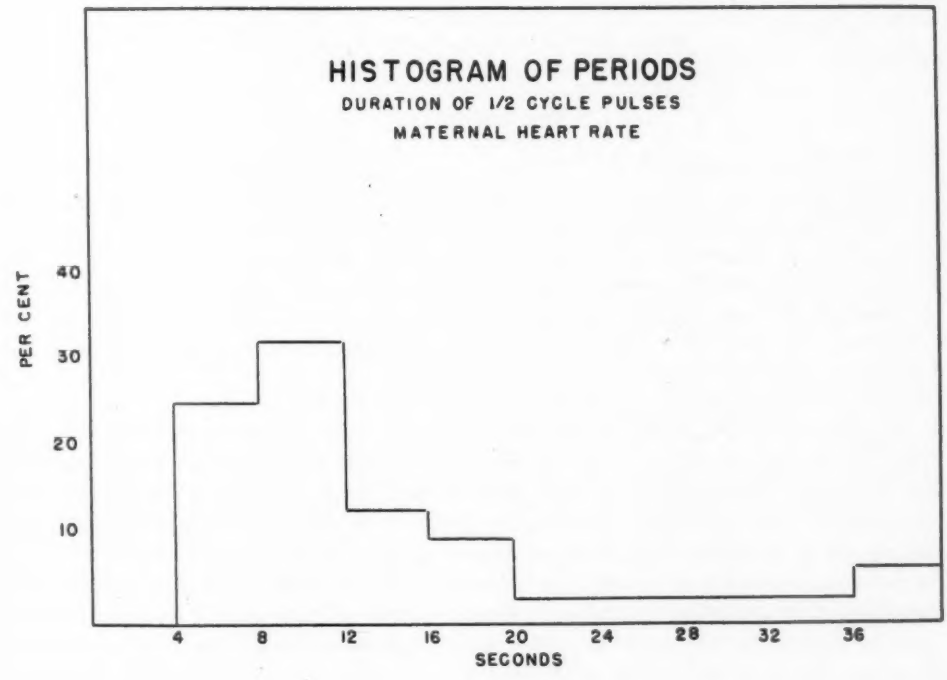
HISTOGRAM OF PERIODS
DURATION OF 1/2 CYCLE PULSES
MATERNAL HEART RATE
PER CENT
40
30
20
10
4
8
12
16
20
24
28
32
36
SECONDS

Fig. 13.

In conclusion, a device to study minute variations in fetal heart rates and rhythms in antepartum patients has been developed. No striking changes have as yet been discovered between normal and abnormal pregnancies prior to labor, but certain normal patterns have been described which give promise of defining one parameter of fetal health in utero. The conventional signs of fetal distress in labor have been studied in a rather large sample. By purification of these data an indication of the very serious prognostic significance of the passage of meconium has been emphasized.

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Discussion

DR. CHARLES E. McLENNAN, San Francisco, Calif. (read by the Secretary).—Although the title of Dr. Hellman's paper implies that he intended to relate fetal heart rate to fetal well-being, my review of his manuscript left me with the feeling that he has not yet had time to come to grips with the heart of his problem other than in a rather speculative fashion. He suggests that his records of fetal heart rates eventually may disclose a normal or standard pattern and that modifications of this pattern ultimately may be correlated with specific unhealthy states in the fetus. Edward Hon in New Haven has suggested similar predictive values for his fetal electrocardiograms, and very recently he has proposed the gathering of additional data by using the electrocardiographic apparatus as a trigger source for a cardiotachometer, which I take to be a device similar to if not identical with that used by Dr. Hellman. Elaborate tools of various sorts have been used for over 50 years in pursuing fetal heart activity, yet there is today very little evidence that these complex machines can outperform the clinician with a stethoscope. Nor am I aware of any indisputable proof that the cardiac rate in utero can predict what the future holds for a particular fetus. But this is not to deny that further investigation might furnish such proof, and certainly Dr. Hellman has made only a meager beginning with his new and perhaps more promising recording device. One must admire his courage in sticking with an apparatus which produces useful records in less than 50 per cent of instances.

Dr. Hellman has looked into the problem of meconium as a sign of fetal distress in labor and concludes that "the passage of meconium carries by far the gravest import for

fetal survival." The perinatal loss associated with meconium was only 8 per cent in single, term, vertex presentations, however. In view of what our most respected textbooks have had to say about meconium and anoxia, this seems to me a rather low figure. If only 2.9 per cent of fetuses show distress in labor, and if only two-thirds of these exhibit meconium, and if only 8 per cent of this latter group winds up in the perinatal loss column, it seems that only rarely will meconium be associated with serious trouble. Perhaps the books should be rewritten in less gloomy terms, particularly one widely used text which distinguishes between "old olive green" meconium—presumably a harmless variety —and the fresh "dark sea green and lumpy" variety which carries a worse prognosis. This authority believes meconium is passed because of active peristalis resulting from hypoxia and likens the situation to the involuntary bowel movement of a drowned or otherwise asphyxiated person. Another author, of equal stature, believes the passage of meconium results from anal sphincter relaxation induced by faulty aeration of the blood. I wonder whether Dr. Hellman would care to comment on these divergent views, particularly in the light of Prystowsky's recent suggestion that fetal heart slowing is related to increased intracranial pressure and impaired medullary blood flow rather than to reduced oxygenation of intervillous space blood. Oxygen gradients in his patients did not correlate at all well with fetal heart rates. In some instances seemingly poor oxygen transfer was associated with a normal rate; in other instances the opposite was true.

But to revert specifically to the material at hand, I think it would be desirable to know much more about the individual instances of perinatal loss following labors in which meconium was passed. Was there anything peculiar or noteworthy about the 17 labors yielding dead babies in the absence of obvious major obstetrical complications, or may we assume that further combing of these records would not be helpful? How long prior to delivery was meconium detected? When was it noted, and was any specific action taken, or is it likely that anything could have been done to change the outcome? And what of the large number of meconium-passers who survived the neonatal period? Are they now as healthy as their fellows who escaped the sea-green bath?

DR. RONALD R. GREENE, Chicago, Ill.—I want to ask two questions: what was the pathology in the case in which meconium was present and no other complication and the baby died? In what percentage of these babies was there premature rupture of the membranes?

DR. E. STEWART TAYLOR, Denver, Colo.—I cannot answer from this presentation the question of what we do with the patient whose cervix is somewhere between 2 and 7 cm. dilated, with a vertex presentation, and who passes meconium-stained amniotic fluid. That is a very difficult problem in which to make a decision. My decision has usually been to be conservative. Most of the time that has been right but sometimes I have regretted it. Do you have anything in your material that would help establish or solidify a policy for the management of such patients?

DR. HELLMAN (Closing).—First, I must say that the electronic development of this apparatus was performed by a group of experts of which I am not one. Long ago I learned not to become involved in this aspect of the problem for its language and conceptions are beyond my abilities. I, therefore, cannot answer any questions regarding the workings of the apparatus.

I tend to agree with Dr. Walker, of Dundee, that the passage of meconium is associated with a reduction in the oxygen of the fetus. Exactly at what level the fetus begins to pass meconium and why is not known. The old explanation of relaxation of the sphincter may or may not be correct.

As for Dr. Greene's question, Dr. Kohl intends to publish a more complete survey of the data which I have just presented. I do not have the information here on whether the membranes were ruptured prematurely. It is quite possible that this was so.

Dr. Taylor's question concerning the treatment of the classic signs of fetal distress is of interest. During the period of this study very little was done to correct the situation except possibly the administration of oxygen to the mother. Very few radical steps were taken and the over-all cesarean section rate for this complication was only 11 per cent. In the meconium group without any obstetrical complications the cesarean section rate was 3 per cent. It is probable that if future work confirms the serious fetal implications of the passage of meconium a more radical approach will be indicated. Both Prystowsky and Hon have independently indicated the variablity of the fetal heart rate and have shown that slowing, particularly in the middle part of labor, may be due to the pressure of the incompletely dilated cervix on the fetal head.

BLOOD VOLUME AND ANEMIA OF MOTHER AND BABY*†

CURTIS J. LUND, M.D., AND THOMAS R. C. SISSON, M.D.,
ROCHESTER, N. Y.

(From the Departments of Obstetrics and Gynecology, and Pediatrics, The University of Rochester School of Medicine and Dentistry)

ANEMIA may be defined in the laboratory as a reduction of hemoglobin and/or red cells per unit volume associated with morphologic changes of these cells. Anemia becomes important physiologically when the total circulating mass of hemoglobin is unable to transport sufficient oxygen to meet tissue requirements.

The accuracy of our common laboratory hemoglobin determination and hematocrit depends upon a constant ratio between plasma and red cell volume. Under conditions of health this relationship is comparatively stable. Many factors are known to alter it, however, such as heat, exercise, hemorrhage, position, altitude, climate, and pregnancy. Failure to understand the nature of volumetric changes in pregnancy has led to the common misconception and misnomer of "physiologic anemia of pregnancy." Anemia is not physiologic, therefore it cannot occur in the normally healthy person whether pregnant or not.

A more precise definition of anemia, we believe, should relate the total hemoglobin mass and red cell volume to the weight of the patient.

Methods

All determinations were done on samples of venous blood collected without stasis. Concentration of hemoglobin was measured by the cyanmethemoglobin method of Crosby, Munn, and Furth.[1] Microhematocrit was determined by the use of an International Hematocrit Centrifuge. Plasma volume was measured by a modification of the method of Nitshe and Cohen[2] with the use of T-1824, and by the extraction of dye in acetone-water as described by Allen.[3] The value of such extraction methods has very recently been re-emphasized by Murray and Shillingford.[4] Experiments yielded 97.0 per cent ± 2.0 per cent recovery on samples in triplicate. From these measurements of plasma volume were derived the values for total blood volume, red cell volume, and total circulating hemoglobin mass. In these calculations the factors for plasma trapped in the hematocrit and for the ratio of body to venous packed cell volume were used.[5, 6, 7] Total circulating hemoglobin mass was calculated from the blood volume and the concentration of hemoglobin. In order to report the data in a fashion common to all subjects, the volumes are presented in milliliters per kilogram of body weight and the hemoglobin mass in grams per kilogram of body weight.

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†Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

More than 130 women were studied serially during one or more pregnancies and the puerperium. Ten had four determinations, 59 were studied three times, and 64 were observed twice during gestation. One hundred and twenty-two full-term infants of these mothers were studied at birth. Of these, 19 were studied serially throughout the first year of life. In addition, single observations were made upon 65 pregnant women and upon 17 healthy nonpregnant women of the same age group. Approximately 90 per cent of our women attended the clinic, the remainder were private patients.

Excluded from consideration were all patients with complications of pregnancy which might obviously affect homeostasis and erythropoiesis, such as cardiovascular disease, infection, hemorrhage, toxemia, renal disease, and others. Included were normally healthy pregnant women without evidence of anemia and women with varying degrees of iron deficiency.

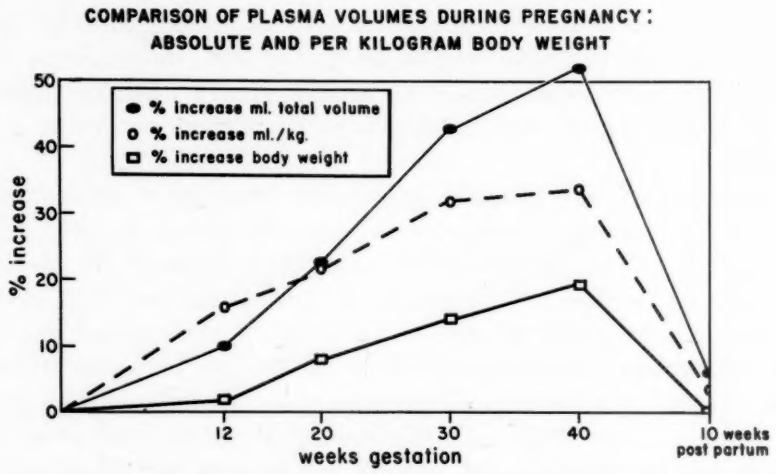

Fig. 1.—Plasma volume in milliliters per kilogram of body weight increases during the first trimester at the time when weight gain is minimal. Although the absolute increase in total plasma volume is greater during late pregnancy, it remains directly proportional to the increased weight.

Results

Plasma Volume.—Most obstetricians are familiar with the changes in total plasma volume which take place during pregnancy. Almost routinely, these have been expressed in terms of volumetric increase in milliliters or in per cent of increase during the course of pregnancy. Little attention has been given to the factor of weight (Fig. 1).

Variation in plasma volume may be due to changes within the individual or it may be due to progressive changes of pregnancy (Fig. 2). The mean plasma volume for 17 nonpregnant women is 52.1 ml. per kilogram. This value is higher than others reported, which varied from 43 to 45 ml. per kilogram.[8, 9, 10] The increase during early pregnancy is rapid, the mean being 60.2 ml. per kilogram. Greater variations within the group are also seen at this time. The maximum volume as well as maximum variability occurs in late pregnancy when the mean is 68.4 ml. per kilogram and extremes of 103.3 and 42.8 ml. per kilogram are noted.

Our repeated observations during gestation suggest that differences in plasma volume are greater between individuals than within an individual. All of the patients (215) in this study, with 409 determinations, were grouped according to their plasma volumes, expressed in milliliters per kilogram of body weight.

In any distribution of values for plasma volume in women, the largest number may be expected to fall within the limits of normal. The remainder of these values will range at a higher or lower level. Two criteria were considered for the classification of plasma volume, the distribution in percentiles and twice the standard deviation. Of these, the percentile distribution fitted the data better, especially in the area of lower values. In the area of higher values the eightieth percentile and twice standard deviation were similar.

Twenty per cent of the women with lowest values were classified as *hypovolemic.* In a similar fashion, 20 per cent of the patients with highest values were designated as *hypervolemic.* The large remainder were considered to be average, and were called *isovolemic.*

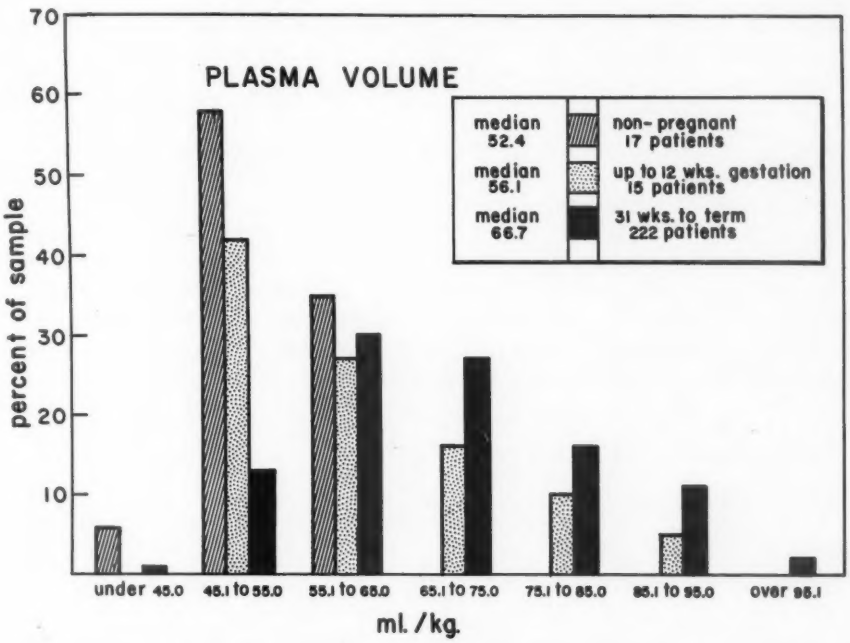

Fig. 2.—The plasma volume of control (nonpregnant) patients was within a narrow, low range. During early pregnancy, many patients were distributed in the higher ranges; and during late pregnancy, over half of the women had values above the highest control. Fifteen per cent showed no increase. Note the similarity between the median and the mean quoted in the text.

It is necessary also to recognize the changes which occur during the progress of gestation. Therefore, criteria for classification must naturally vary according to the period of gestation at which the observation is made. Table I shows these values for the nonpregnant woman as well as those observed throughout gestation.

TABLE I. A CLASSIFICATION OF PLASMA VOLUME (MILLILITERS PER KILOGRAM) AT DIFFERENT PERIODS OF GESTATION

WEEK OF GESTATION	NO. OF PATIENTS	HYPOVOLEMIA	ISOVOLEMIA	HYPERVOLEMIA
Not pregnant	17	50.0	<45.0-55.0	>55.0
Under 13	19	45.0	<50.0-65.0	>65.0
13-20	49	50.0	<50.0-70.0	>70.0
21-30	102	55.0	<55.0-80.0	>80.0
Over 30	222	55.0	<55.0-85.0	>85.0

Eighteen patients with hypervolemia and 15 patients with hypovolemia at term had been studied repeatedly during pregnancy. Table II shows that hypervolemia was maintained throughout individual pregnancies with few exceptions. This table also records the individual observations of 15 mothers who were hypovolemic at term. Nearly all of them showed hypovolemia or low isovolemia during earlier pregnancy.

TABLE II. INDIVIDUAL VARIATION OF PLASMA VOLUME (MILLILITERS PER KILOGRAM) DURING PREGNANCY IN PATIENTS WHO ARE HYPERVOLEMIC OR HYPOVOLEMIC AT TERM

PATIENT	WEEK OF PREGNANCY				
	13	13-20	21-30	31-34	>34
Hypovolemia.—					
PO 1			45	44	51
PO 2	39	48		46	
PO 3		51	51		46
PO 4			76	64	47
PO 5			65	47	49
PO 6			57	60	48
PO 7			51		48
PO 8			75		48
PO 9			48	50	57
PO 10		56	64	58	51
PO 11		54		54	52
PO 12		62			53
PO 13			59	54	53
PO 14			62		53
PO 15				54	55
Hypervolemia.—					
PE 1			86	103	87
PE 2	65	67		102	95
PE 3	70	84		102	
PE 4		89			96
PE 5	75		77	87	94
PE 6	80	74	93		96
PE 7			91	93	95
PE 8				94	128
PE 9		85	84	93	
PE 10		84	76		91
PE 11				90	89
PE 12			76		89
PE 13			85	88	76
PE 14		69		80	88
PE 15		60	74		88
PE 16			87	103	80
PE 17			86		86
PE 18		86		86	

To test further uniformity, analysis was made of 133 mothers studied more than once during pregnancy. Only a third (42 patients) were observed to change their classification. Although some patients moved from one class to the next, major individual variations were infrequent, and we never observed a mother to change from hypervolemia to hypovolemia or the reverse.

Total Hemoglobin Mass and Red Cell Volume.—It has been repeatedly demonstrated[11, 12] that the total red cell volume and hemoglobin mass increase during normal pregnancy. Although this fact has been recognized for many years, the significance and value of such measurements have been generally neglected in the study of pregnant women. Except in unusual circumstances, the values for hemoglobin mass and red cell volume parallel each other and have similar significance. For this reason they will be described together.

There is little comparative data available which might establish "normal" values for healthy nonpregnant women, and values for healthy pregnant women have received little attention. In order to provide comparative standards within our laboratory, we have studied both. Seventeen nonpregnant women of childbearing age were studied during the midmenstrual cycle. They were in good health and exhibited neither clinical nor laboratory evidence of anemia (Table III).

TABLE III. COMPARISON OF PERIPHERAL AND VOLUMETRIC MEASUREMENTS OF RED CELLS AND HEMOGLOBIN IN 17 NORMAL NONPREGNANT WOMEN

	TOTAL (BODY)		VENOUS	
	RCV (ML./KG.)	HEMOGLOBIN MASS (GM./KG.)	HEMATOCRIT (%)	HEMOGLOBIN (GM./100 ML.)
Mean	28	10.0	40	12.5
High	34	11.8	45	14.0
Low	24	8.8	35	11.5

In our opinion acceptable values for circulating hemoglobin mass and total red cell volume in pregnancy most certainly should be no lower than those of the normal nonpregnant woman. To test the validity of this opinion, 8 semiprivate and private patients were studied. They were chosen at random. Their diets were unquestionably adequate, and their pregnancies uncomplicated. Table IV shows the mean hemoglobin mass was 12.3 Gm. per kilogram, and the total red cell volume 33.9 ml. per kilogram. No value for hemoglobin mass fell below 11.0 Gm. per kilogram, and no value for red cell volume fell below 29.0 ml. per kilogram. We believe that anemia is not present in pregnancy as long as these levels are equaled or exceeded.

TABLE IV. COMPARATIVE PERIPHERAL AND VOLUMETRIC STUDIES OF 8 NONANEMIC WOMEN IN LATE PREGNANCY

	PV (ML./KG.)	RCV (ML./KG.)	HEMOGLOBIN MASS (GM./KG.)	HEMOGLOBIN (GM./100 ML.)	PCV (%)	SERUM IRON (μG %)
N 1	96.2	39.8	15.1	11.1	33.5	101
N 2	96.4	32.7	11.4	8.8	29.0	106
N 3	85.9	37.8	13.0	10.5	35.0	133
N 4	77.3	33.4	12.5	11.3	34.5	111
N 5	73.9	31.2	11.1	10.6	34.0	142
N 6	61.1	31.0	11.9	12.9	38.5	111
N 7	74.2	34.1	12.8	11.8	36.0	100
N 8	73.9	31.2	11.0	10.5	34.0	82
Mean	79.9	33.9	12.3	10.9	33.2	111

Statistical methods cannot be applied to the determination of normal values in a clinic population where the incidence of iron deficiency anemia is high. By applying the normal values to a distribution curve of all patients studied, however, it was possible to establish a classification for borderline and severe anemia (Table V).

TABLE V. PROPOSED STANDARDS FOR LABORATORY DIAGNOSIS OF ANEMIA IN LATE PREGNANCY

		TOTAL HEMOGLOBIN MASS (GM./KG.)	TOTAL RED CELL VOLUME (ML./KG.)
Normal (not anemic)	above	11.0	29.0
Borderline anemia	between	11.0-9.5	29.0-0.25
Severe anemia	below	9.5	25.0

Studies of Infants

Laboratory methods to assess the hematologic state of newborn infants were the same as for their mothers. Sixty-six normal babies were studied in the first 3 to 5 days of life after shifts in the plasma compartment had become stabilized.

Plasma Volume.—A wide range of plasma volume was noted in all infants, but the values were at a higher level in the babies of nonanemic mothers. The frequency distribution of individual plasma volumes shows that extreme deviation from the mean of 61 ml. per kilogram occurs (Fig. 3). Five infants in this study were considered to be hypervolemic, with a volume greater than 75 ml. per kilogram.

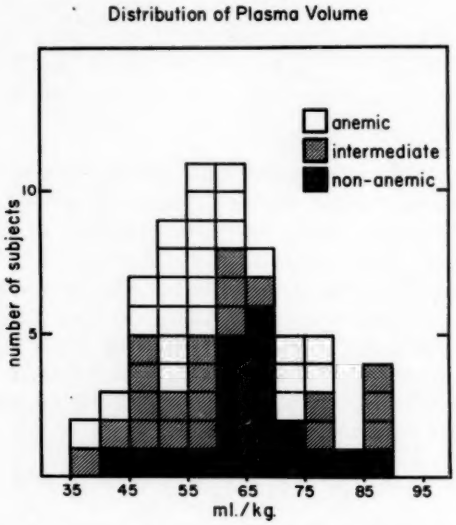

Fig. 3.—Note the distribution of plasma volumes in babies of nonanemic mothers, and in those with borderline and severe anemia. The wide range of values from the mean of 61 ml. per kilogram is shown in all groups. (From Sisson and Lund: Am. J. Clin. Nutrition 6: 376, 1958.)

TABLE VI. MEAN VALUES FOR PERIPHERAL AND VOLUMETRIC BLOOD STUDIES AND SERUM IRON OF MOTHERS AND THEIR NEWBORN INFANTS

	NO. PATIENTS	HEMOGLOBIN (GM./100 ML.)	PCV (%)	HEMOGLOBIN MASS (GM./KG.)	RCV (ML./KG.)	SERUM IRON (μG %)
Mothers.—						
Not anemic	21	11.2	34.7	12.5	33.6	80
Borderline	20	10.2	33.4	10.2	28.6	61
Severely anemic	25	9.9	31.6	7.9	22.0	67
Infants.—						
Not anemic mothers	21	16.8	49.7	18.9	46.2	93
Borderline mothers	20	16.5	50.0	16.9	42.4	84
Severely anemic mothers	25	15.7	47.2	15.1	37.3	89

Total Red Cell Volume.—Babies of nonanemic mothers had a mean total red cell volume of 46.2 ml. per kilogram. The mean value in babies of mothers with borderline anemia was 42.4 ml. per kilogram, and in babies of severely anemic mothers was 37.3 ml. per kilogram. The mean difference of 8.9 ml. per kilogram between the highest and lowest groups is a significant amount (Table

VI). The magnitude of those differences is similar to that of the mothers. They indicate that anemia in the mother is frequently followed by a distinctly reduced total red cell volume in the newborn.

Total Circulating Hemoglobin Mass.—The infants of nonanemic mothers had an average total hemoglobin mass of 18.9 Gm. per kilogram, babies of the mothers with borderline anemia had values of 16.9 Gm. per kilogram, nearly 10 per cent lower. Babies of the severely anemic mothers had an average of 15.1 Gm. per kilogram. This is 20 per cent lower than the normal group and represents a loss of 20 per cent of the iron otherwise available to these infants for hemoglobin synthesis in later months of life.

Discrepancies between hemoglobin concentration and total hemoglobin mass, and between venous hematocrit and total red cell volume in a few instances are due to very low or very high plasma volumes. This duplicates such paradoxical values found in pregnancy and other conditions.

A detailed analysis of the data obtained from a study of the infants of these mothers has been published.[13]

Placental Transfusion.—In the study of blood volume of the newborn it is necessary to consider the effect of the so-called "placental transfusion." This is said to occur as a result of delayed ligation of the umbilical cord. A transfer of placental blood mass, said to exceed 60 ml. by some investigators,[14, 15, 16] would add an appreciable amount to the red cell volume and iron reserve of the infant.

In order to determine the effect of delay in cord clamping, the blood volumes of 38 babies were determined on the first and third days of life following immediate or delayed ligation.[17] A group of babies were similarly studied after "milking" of the cord. It was concluded that neonatal blood volumes are, in general, not related to techniques of cord ligation unless artificial drainage of the placental blood mass is employed.

Comment

Under conditions of stable blood volume, the ordinary laboratory determinations of hemoglobin, red blood cell count, and hematocrit (packed cell volume) are subject to error as great as or greater than is the estimation of total plasma and red cell volume. However, under conditions known to produce extensive changes in blood volume such as occur in pregnancy, hemorrhage, and the puerperium, the peripheral blood determinations may give an inaccurate, misleading evaluation of the intravascular content (Fig. 4). There is no longer any doubt, in our opinion, about the significance and reliability of the principles used in this study. Williams and Parsons[18] have recently said, "It seems paradoxic that such a small margin of error in blood volume [± 3 per cent in our laboratory] should precipitate such a controversy when the accuracy of red blood cells, hemoglobin, and hematocrit have gone without serious question for so many years."

Calculation of total blood volume need not be a formidable or expensive procedure. There are several acceptable direct and indirect methods of measurement. Our experience with T-1824 (Evans blue) continuously for a period of 10 years has convinced us of the reliability of this method.

The magnitude of the variation in plasma volume clearly indicated the false impression which may be gained if hemoglobin and red cell volume are

expressed as in the common laboratory determination of hemoglobin concentration and hematocrit (PCV) (Fig. 5). Because of this, we believe the estimation of hemoglobin in relation to body weight is more accurate and meaningful.

Beyond this our data show the fallacy of estimating total blood volume from the standard tables derived from the weight. Table VII compares 2 patients of similar weight with identical hemoglobin concentration and hematocrit values at term. In spite of this superficial similarity, patient PE 1 had twice the blood volume and hemoglobin mass of patient PO 7.

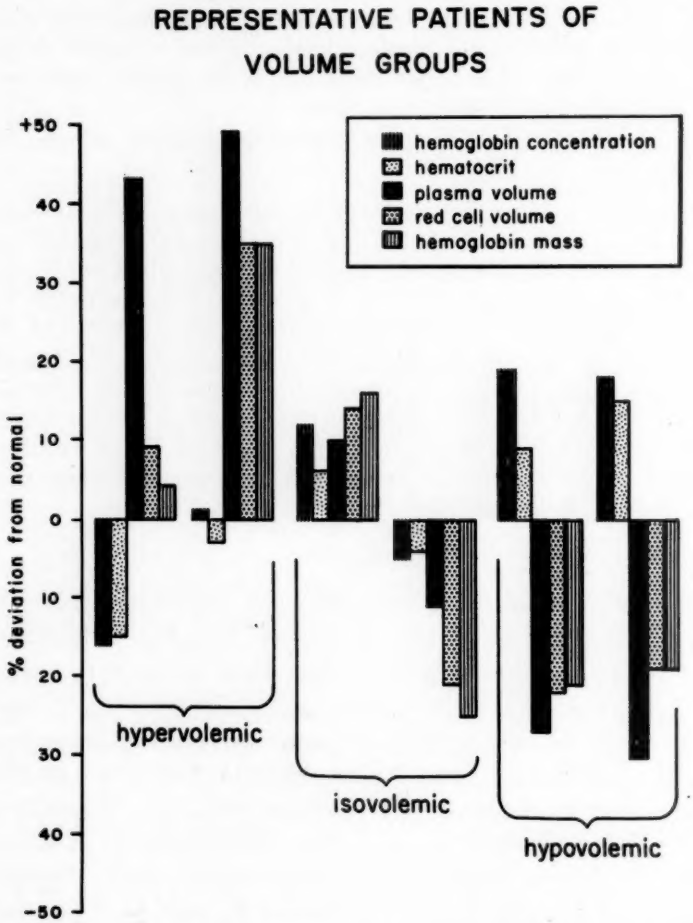

Fig. 4.—In hypervolemia and hypovolemia blood counts may be misleading by deviating in a direction opposite the total mass. In isovolemia these values usually parallel each other.

At present there is no satisfactory explanation for the volumetric changes of pregnancy. Although the greatest absolute increase in blood volume occurs during the last half of pregnancy it is proportionate to the gain in weight, and might be expected. By contrast, the greatest increase in plasma volume per kilogram of body weight occurs during the first trimester when weight gain is the least (Fig. 1). As this change antedates the formation of the placenta and the large arteriovenous shunt which it provides, we believe that the stimulus for expansion of plasma in early pregnancy has another origin.

The stimulus for the increase in total hemoglobin mass and red cell volume is equally obscure. Increases in plasma volume were usually, but not always, accompanied by increases in hemoglobin mass (Fig. 6). Some patients with hypervolemia did not have a commensurate rise in hemoglobin mass which, we believe, was due to a deficiency of iron. In sharp contrast to the hypervolemic state, hypovolemia was consistently associated with a low hemoglobin mass and red cell volume. This finding also suggests that an expanding plasma volume

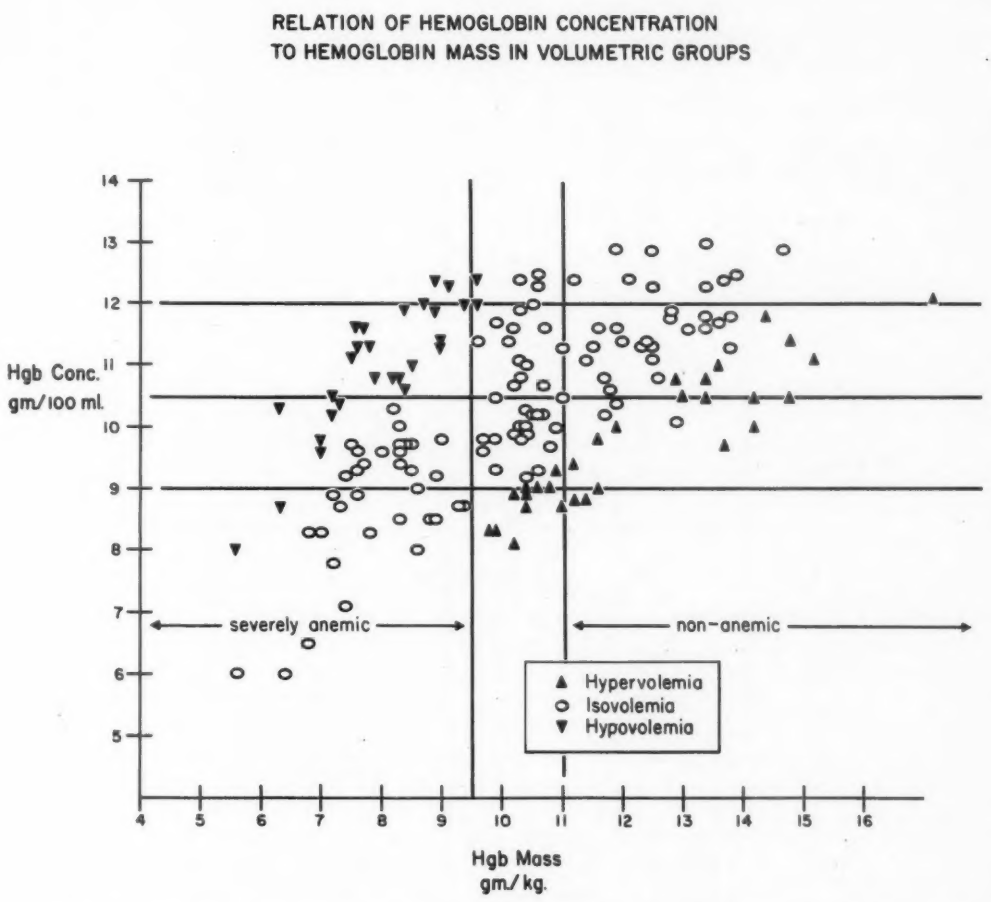

Fig. 5.—Anemia may be present when the hemoglobin concentration is above 12 Gm. per 100 ml. Conversely, patients may not be anemic with hemoglobin concentrations below 10 Gm. per 100 ml.

provides a hematopoietic stimulus. The anemia associated with hypovolemia cannot be recognized without blood volume measurements, as studies of peripheral blood were almost uniformly within the so-called normal limits for pregnancy.

The value and application of volumetric methods are not limited to the mother. Blood volume measurements in the infant are useful in assessing hemolysis, blood loss, and anemia. We[19] have previously reported the advantage of such techniques in exchange transfusion therapy for hemolytic disease of the newborn.

TABLE VII. COMPARISON OF THE PERIPHERAL AND VOLUMETRIC BLOOD STUDIES OF 2 WOMEN IN LATE PREGNANCY

	PATIENT PE 1	PATIENT PO 7
Weight (kg.)	58.0	59.6
Hemoglobin (Gm./100 ml.)	10.5	10.5
Hematocrit (PCV) (%)	33.0	33.0
Total blood volume (ml.)	8,166	4,066
Total hemoglobin mass (Gm.)	856	427
Total hemoglobin mass (Gm./kg.)	14.8	7.2

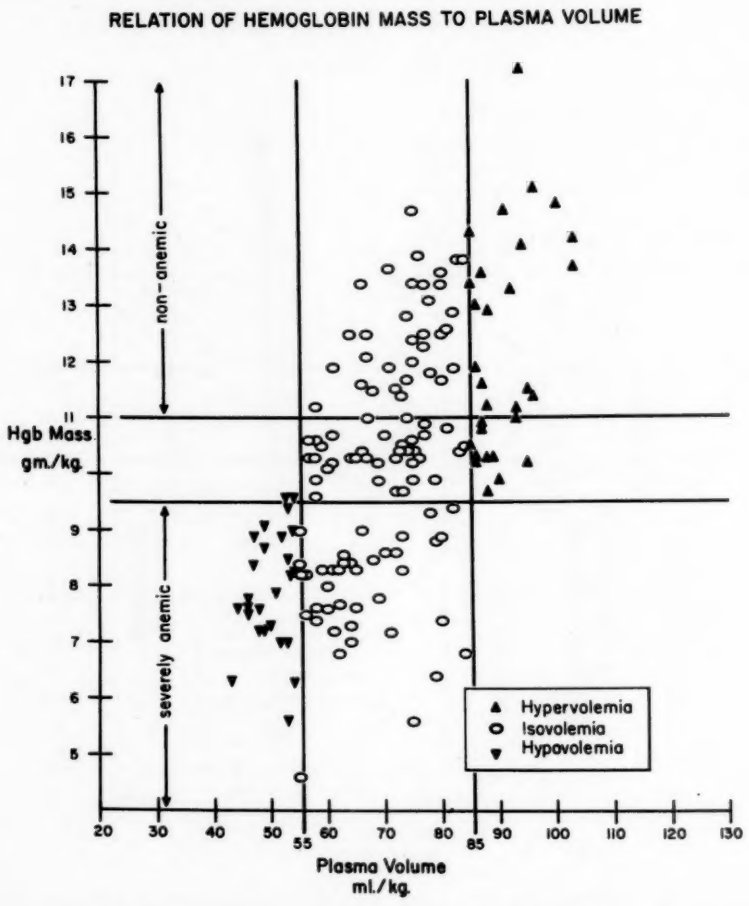

Fig. 6.—Patients with hypovolemia uniformly exhibit a low hemoglobin mass. Most patients with hypervolemia have a high hemoglobin mass. Great variability is noted in patients with isovolemia.

The hemoglobin mass of the healthy full-term infant of a nonanemic mother may be expected to be reduced by half (8-10 Gm. per kilogram) during the first 9 weeks of life and the iron released from the destroyed erythrocytes is the chief source of this element for the production of new hemoglobin. Although peripheral values may appear normal, our data indicate that infants of anemic mothers have a smaller hemoglobin mass than do infants of normal mothers. Under these conditions the iron released during early neonatal life is insufficient to meet the hematopoietic demands of the first half year of life and such babies do have an iron deficiency anemia.

It is obvious that determinations of blood volume are not needed on every pregnant woman. It is equally obvious that such determinations are important in the study of the anemias of pregnancy, or in assessing response to therapeutic agents. The physician will find such information of value in the management of patients with heart disease, toxemia, diabetes; and it would be of great help where shock, hemorrhage, and traumatic delivery are anticipated.

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Discussion

DR. ARTHUR B. HUNT, Rochester, Minn.—Drs. Lund and Sisson's mean value for plasma volume per kilogram of body weight is 52.1, which is higher than that found by other workers they quote, namely, 43 to 45 ml. per kilogram. It is possible there are other normal values than those quoted that are even lower, that is in the range of 40 ml. per kilogram. There is, therefore, an appreciable difference of from 13.5 to perhaps 24 per cent here for normal values, which, if it is an error, is quite a large one. Such sources of error as the plasma trapped in the red cell column of the hematocrit and the bodily hematocrit error were corrected for by the authors. I wonder if Dr. Lund cares to comment on these higher values among nonpregnant women. I doubt if this difference invalidates the results in pregnancy as the same techniques were used in the 17 nonpregnant patients as were employed in his hundreds of determinations during pregnancy.

In the comment, Dr. Lund quoted a paper published this year to the effect that it was paradoxical that the accuracy of red blood cell count has gone without serious question for so many years. Twenty years ago, however, Magath and Berkson did point out the inaccuracy of red cell counts and indeed their work has caused some major medical centers to abandon them. Incidentally, the error of the red cell count is in the range of plus or minus 8 per cent and is higher than that of the determination of hemoglobin concentration of the hematocrit.

There is virtue in Dr. Lund's making serial studies in each of his patients in most instances (which has not always been observed by others) rather than spot determinations at one moment in pregnancy. This allows him to tell us that his work suggests that differences in plasma volume are greater between individuals than within an individual.

The authors, applying their volumetric methods to infants, found correlation in iron nutrition between the mother and the newborn infant after birth. Previous work on this point has been at variance as to whether the fetus shares constantly its mother's state of iron nutrition. Some have long regarded the fetus as the notoriously good parasite. As for

as I know, however, there have been no studies of plasma volume (with hemoglobin mass per kilogram calculated) brought to this problem. The authors may have settled the question in favor of the premise that the baby does partake of its mother's hemoglobin and iron status. In any event, the important and fundamental contribution of this paper seems to be that one cannot finally measure the hemoglobin of an obstetric patient accurately without plasma volume determinations and the calculation of the crucial figure of hemoglobin mass in grams per kilogram of body weight.

Drs. Lund and Sisson have shown us for the first time, I believe, why some patients in apparently good condition as to hemoglobin by ordinary tests can ill afford to lose blood. None of the one fifth of the hypervolemic patients evidenced severe anemia but most of the hypovolemic patients did. About one third of the isovolemic patients had appreciable anemia. These, then, are the ones in danger from even moderate hemorrhage. We do not have the final proof that they did withstand bleeding poorly. I should like to ask Dr. Lund if any of the hypovolemic women sustained hemorrhage and, if so, how they fared. The group was rather small and perhaps, therefore, experienced no test of blood loss.

This paper and a previous one by Dr. Lund, as well as the several contributions by Holly and others, should bury the idea of a so-called physiologic anemia of pregnancy and make us realize that it is possible to demonstrate that this type of anemia in pregnancy is an iron deficiency. Given available iron, the hematopoietic system will produce normal levels of hemoglobin in spite of the increasing plasma volume in pregnancy. Therefore, by way of practical therapy it is obvious that all these patients need is cheap ferrous iron (possibly with cobalt), and expensive liver products, vitamins, folic acid, and the like are ineffective.

It is regrettable, but no fault of the authors, that most obstetric patients cannot have the prognostic and, therefore, the therapeutic advantage of determination of their hemoglobin mass per kilogram of body weight in pregnancy. But the test of plasma volume, as Dr. Lund notes, need not be formidable or expensive in patients who have anemia, organic disease, or in whom more than average obstetric hemorrhage may be expected. We are reporting in the near future, for example, that blood loss in cesarean section is greater than is ordinarily appreciated and that such patients could well have the advantage of volumetric studies.

Only in favored areas do factors such as cancer and heart disease lead in maternal deaths. In much of the country hemorrhage is still the leading cause of maternal mortality. That is the final reason why this thorough study, offering new and more accurate ways of assaying the pregnant woman's hemoglobin assets or liabilities, is so important.

DR. LUND (Closing).—The difference between the plasma volume observed by others and by us has caused us some concern. We have checked our results and I have no explanation for it other than laboratory variability.

Actually I think our 17 normals constitute a greater number than has been described, as the number of normal women studied is very small.

Dr. Hunt's second question about hypovolemia and hemorrhage is important. In our department we classify obstetric hemorrhage as loss of 10 per cent of the circulating blood volume. We realize how inaccurate this estimate may be, but we still believe it is better than using an arbitrary figure. I think 20 per cent loss of blood volume constitutes serious hemorrhage and produces shock. Therefore, the patient with hypovolemia will be subject to greater effects from hemorrhage.

I would like to say one final word about the most unusual patient of our study recorded the day before I left. She had congenital heart disease with Marfan's syndrome, weighed 140 pounds. Her total blood volume was 9,200 c.c. on two occasions and her hemoglobin was 8.4 and 7.7 Gm., and a group of visiting physicians could not possibly understand how we could say that she was not anemic. She had a hemoglobin mass of 12 Gm. per kilogram and she was not anemic. Although there was some talk of transfusing her, I think you would agree that a woman with this total blood volume should not be transfused. In fact it might dangerously overload an already failing heart.

A THIRTEEN-DAY HUMAN OVUM STUDIED HISTOCHEMICALLY*†

ARTHUR T. HERTIG, M.D., ELEANOR C. ADAMS, B.A.,
DONALD G. MCKAY, M.D., JOHN ROCK, M.D.,
WILLIAM J. MULLIGAN, M.D., AND MIRIAM F. MENKIN, M.A.,
BOSTON AND BROOKLINE, MASS.

(From the Departments of Pathology and of Gynecology, Harvard Medical School, The Free Hospital for Women, and the Department of Embryology, Carnegie Institution of Washington)

THE purpose of this communication is to describe some histochemical observations on a normal human fertilized ovum recovered at hysterectomy during its fourteenth day of development. This specimen lies within Horizon VI of Streeter,[1] possessing primitive unbranched villi and a definitive yolk sac. From its gross appearance, size, and embryologic development, it is most comparable to two Carnegie specimens, No. 8672 and No. 8360.[2] It is slightly younger than early villous ova such as Carnegie No. 7801, possessing a definitive yolk sac,[3] and slightly older than Carnegie No. 8330, possessing a primordial yolk sac.[2] Hence the present specimen‡ is the youngest thus far seen possessing a definitive yolk sac. In reference to other specimens in the literature, it is comparable to the Linzenmeier ovum[4] and slightly younger than the Yale (Ramsey[5]) ovum of 13 plus days, Carnegie No. 6734, the Peters[6] ovum of 14 days, and the Edwards-Jones-Brewer[7] ovum of 15 to 16 days' developmental age. An outline drawing showing human ova of 11 to 15 days of age or in their twelfth to sixteenth day of development may be seen on page 149 of Hertig and Rock's[8] article on ova of the previllous stage.

In order to orient this specimen in the whole spectrum of human development, Horizon V is characterized as an ovum implanted but still avillous; Horizon VI as primitive villi, distinct yolk sac; and Horizon VII as branching villi, axis of germ disc defined.[1]

Horizon VI is described more in detail as follows,[2] "At 13 days of age the ovum is evident upon gross examination by virtue of its size and elevation. It is not completely embedded within the endometrium. Bleeding, which may occur

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‡This specimen has been presented at the April, 1957, meeting of the American Association of Anatomists in Baltimore and therefore has been published in abstract form only.[9] One picture comparable to Fig. 4 has already been submitted for publication by McKay and associates[10] in their paper on the histochemistry of normal trophoblast throughout pregnancy and by one of us (A. T. H.[11]) in an address before the Central Association of Obstetricians and Gynecologists at their 1957 meeting in Omaha, Nebraska. The present communication is the only complete description of the specimen prepared for publication.

through the unhealed implantation site, arises from the increased flow of maternal blood into the lacunar spaces resulting in rupture of the thin-walled abembryonic pole of trophoblast. There is also bleeding into an occasional endometrial gland whose walls have been eroded by invading trophoblast thereby allowing the maternal blood in the lacunar spaces to flow into the gland lumen. Since this bleeding occurs at about the time of the first missed menstrual period it is an occasional source of clinical inaccuracy in foretelling the date of expected delivery.

"In the trophoblastic shell of the ovum, the cytotrophoblastic cells are rapidly proliferating so that their total mass now exceeds that of the syncytium. The latter now lines the intervillous space which contains occasional streamers of projecting syncytiotrophoblast. Some syncytium detaches as it invades the endometrial stroma and is seen as giant cells within the decidua. Primitive villi are now forming. They have a shallow core of mesoblasts and angioblasts arising from and projecting into otherwise solid cytotrophoblast which in turn is covered by syncytium. The peripheral tips of the villi are coalescing to form the cytotrophoblastic placental floor which is perforated by capillary sinusoids supplying the intervillous space with maternal blood.

"The mesoblast which lines the chorion is more abundant than in earlier stages particularly in the space between the amnion and the chorion. Angioblastic tissue, also delaminating from cytotrophoblast and lying in this mesoblast, is in various stages of early differentiation."

The definitive yolk sac is present in this developmental stage. The mechanism of its formation from the larger exocelomic cavity found in 12 day ova is not well understood. Presumably, however, the remnants of the ruptured Heuser's membrane (primordial yolk sac or the exocelom surrounded by Heuser's membrane) adjacent to the endoderm join to form a smaller vesicle.*

Material and Methods

The *clinical history* of the patient, and gross and microscopic description of the uterus, tubes, and ovaries follow.

The patient, Mrs. A. B., married 12 years, F. H. W. No. 58105, was a 32-year-old gravida vi, para vi, with 5 living children. Her menstrual history revealed the menarche at the age of 12 years, with periods at intervals varying from 28 to 30 days, and with some discomfort but without intermenstrual staining. Her chief complaint was a severe bearing down sensation, worse with periods, apparently due to cystocele, rectocele, and first-degree prolapse, and complicated by urinary incontinence. It was decided to treat the patient by plastic repair of the pelvic supporting tissues followed by a hysterectomy. Accordingly, on the twenty-ninth day of the menstrual cycle, following two previously recorded cycles of 29 days, an anterior colporrhaphy, perineorrhaphy, total hysterectomy and bilateral salpingo-oophorectomy were performed. Coital dates were recorded 14 and 15 days prior to operation.

The *description of the surgically removed material,* F. H. W. S-54-3305:

The specimen which was received unopened from the operating room immediately upon removal consisted of a complete uterus with both tubes and ovaries. The uterus measured

*Since the preparation of this manuscript, our attention has been called to a paper appearing in Acta anat. 30: 656, 1957, by F. Rossi and E. Reale entitled, "The Somite Stage of Human Development Studied With the Histochemical Reaction for the Demonstration of Alkaline Glycerophosphatase."

8.5 by 6.5 by 4 cm. The serosal surface was normal. There were healed bilateral cervical lacerations with a stellate laceration anteriorly. The uterine cavity measured 4.3 by 3.7 cm. On the posterior wall near the right cornu, 0.5 cm. from the fundus and 0.5 cm. from the laterally opened margin, there was a small nodule about 0.15 cm. across and with surrounding fine hemorrhagic beading. This represented an early implanted fertilized ovum about 12 to 13 days of age. The remainder of the endometrium was pale except for petechial hemorrhages and it averaged 0.4 cm. in thickness. The implantation site with the ovum was fixed in cold acetone within 5 minutes of receipt of the specimen in the laboratory. The implanted ovum measured 1.64 mm. after such fixation. This size indicates that it is somewhat bigger than Carnegie No. 7700 (a 12½ day specimen). The myometrium measured approximately 2 cm. in thickness. The right tube measured 7.5 cm. and was patent at the fimbriated end. There were two accessory ostia, that is, three ostia in all. The right ovary measured 3.5 by 3.5 by 2.5 cm. It contained a corpus luteum 2 cm. in diameter with thick, convoluted, 0.3 to 0.4 cm. gray-brown active-appearing border and small red central coagulum. There were many developing follicles at the periphery with hemorrhage. The left tube measured 7 cm. The ostium was patent. There were fine fibrous fimbrial adhesions. The left ovary measured 3.5 by 2 by 1.8 cm. It was pale and contained follicles without hemorrhage. Microscopic diagnoses based on 5 hematoxylin and eosin stained slides were as follows: Chronic cervicitis with epidermidization and squamous metaplasia; early pregnancy, 12 to 13 days; early adenomyosis of the myometrium; negative tubes with hydatid of Morgagni, Walthard cell rest, and accessory ostia, right tube; active corpus luteum of pregnancy with cystic follicles and stromal endometriosis of ovary.

Preparation of the Specimen.—The ovum, the thirty-fourth of those found in a series of 211 Hertig-Rock cases[2] was assigned as Fetus No. 55 in a series of human embryos of all stages of gestation collected in this laboratory for histochemical study.[12, 13] A small block of endometrium containing the ovum was fixed in ice-cold acetone and dehydrated at –20° C. for 48 hours. It was then brought to room temperature and cleared overnight in oil of cedarwood. After a 1 hour bath of xylol, it was infiltrated in 52 to 54° C. tissue mat for 3 hours. The paraffin block was stored in the freezer (–20° C.) until cut.

The entire block was cut serially at 7μ and the sections were mounted according to the method used in this laboratory for the study of small (up to 13 mm.) human embryos (Fig. 1). Dry ribbons of 20 sections each were arranged in parallel rows on black tissue paper. The first section in each row (Section 1, 21, 41, 61, etc) was mounted in series on a 3 by 2 inch slide for the first reaction (alkaline phosphatase). The second section of each row, Section 2, 22, 42, 62, 82, etc., was then mounted on the second slide (H. and E.). When all the sections had been thus mounted, each slide contained seriated sections in intervals of every twentieth section. From study of the dimensions of other ova in this stage of development, it had been calculated that this interval of seriated sections would ensure that each of the 20 slides would contain at least one section passing through the germ disc area.

The sections were mounted on clean albuminized slides and were spread on a thin film of distilled water with the exception of the sections for glycogen, glycoprotein, and RNA, which were spread on a film of absolute ethyl alcohol. The slides were then drained, dried overnight at 37° C., and stored at –20° C. until reacted or stained.

Histochemical Reactions

Alkaline Phosphatase.—Azo dye method (Manheimer and Seligman[14]) was used, with Fast Blue 2B (National Aniline Dye Corp.) as a coupling agent, with incubation at room temperature for 1 hour at pH 9.4. As a control a slide was incubated in a similar mixture with the substrate omitted. The slides were lightly counterstained with hematoxylin.

Acid Phosphatase.—Azo dye method (Burton[15]) was used, with Fast Blue 2B as a coupling agent, with incubation at 37° C. for 2 hours at pH 5.8. As a control a slide was incubated in a similar mixture with the substrate omitted.

Adenosine-5-Phosphatase and Glycerophosphatase pH 7.5 (Pearse and Reis[16]).—The slides were not deparaffinized and were incubated at 37° C. for 3 hours. A control slide was incubated in a similar mixture with the substrate omitted. The glycerophosphatase pH 7.5 is considered an added control to indicate the specificity of the adenosine-5-phosphatase reaction. A light hematoxylin counterstain was used.

Glycogen and Glycoprotein.—The slides were postfixed in Rossman's fluid and stained according to the periodic-acid-Schiff method of McManus.[17] The glycoprotein slide was saliva digested before staining.

Ribonucleoprotein.—The slides were postfixed in Zenker's fluid and stained with eosin-methylene blue. A control slide was digested with ribonuclease before staining.

The slides stained with hematoxylin and eosin and with phosphotungstic acid-hematoxylin were postfixed in Bouin's fluid. Although acetone produces considerable cytological distortion, it was chosen as a fixative in order to preserve maximal enzymatic activity and glycogen following paraffin infiltration.

Description of Specimen

Age of Ovum.—On the basis of its size, morphology, clinical history, and comparison with other specimens within this developmental horizon, the ovum is about 13 days of age or in its fourteenth day of development.

Size.—The dimensions of this early villous ovum after paraffin embedding and serial sectioning are as follows: over-all ovum, 1.77 by 1.33 by 0.598 mm.; chorionic cavity, 0.73 by 0.68 by 0.221 mm.; embryo including amnion and yolk sac, 0.196 by 0.315 by 0.076 mm.; germ disc alone, 0.196 by 0.296 by 0.044 mm. These dimensions are comparable to those of previously reported specimens lying within Horizon VI.[2]

General Features of the Endometrium.—The upper two thirds of the endometrium shows moderate-to-advanced progestational hyperplasia characterized by slight-to-moderate predecidua, prominent and somewhat dilated spiral arterioles, marked stromal edema, and active glandular secretion. The basal one third shows dense stroma and quiescent glands. Immediately beneath the ovum the glands contain inspissated secretion due to blockage by the ovum whereas elsewhere the secretion is finely granular. In one instance, the gland beneath the ovum contains recent hemorrhage (Fig. 9) due to communication of the lumen of the gland, eroded by the trophoblast, with the blood-filled intervillous space. Such hemorrhage into the gland is normal for this stage of gestation and is comparable to the hemorrhage from the abembryonic pole of the ovum which is also normal in later stages of Horizon VI. This surface hemorrhage plays a part in the formation of the *Schlusscoagulum* originally described by Peters[6] in 1899. The present specimen has a small surface coagulum without, however, much hemorrhage (Figs. 2, 3, 4, 5, and 9).

General Features of Ovum.—This is a normally but somewhat shallowly implanted early villous ovum (Figs. 2-9) possessing a bilaminar germ disc (Figs. 10-12) apparently without axial differentiation but possessing a well-developed amnion and a very recently formed definitive yolk sac. Remnants of Heuser's or the exocelomic membrane, the wall of the primordial yolk sac, are present in the chorionic cavity. The chorionic mesoblast is well formed but the chorionic villi are still essentially solid epithelial structures with the earliest suggestion of mesoblastic core formation continuous with the chorionic mesoblast. Angiogenesis in the form of solid multicellular strands is just beginning.

The intervillous space is well formed but many lacunae of various sizes are present within the syncytiotrophoblast and are not yet incorporated into the intervillous space (Fig. 14). The latter contains clotted and unclotted maternal blood, together with the contents of a recently eroded endometrial gland (Figs. 22-25) presumably a source of nutrition for the early ovum. The syncytiotropho-

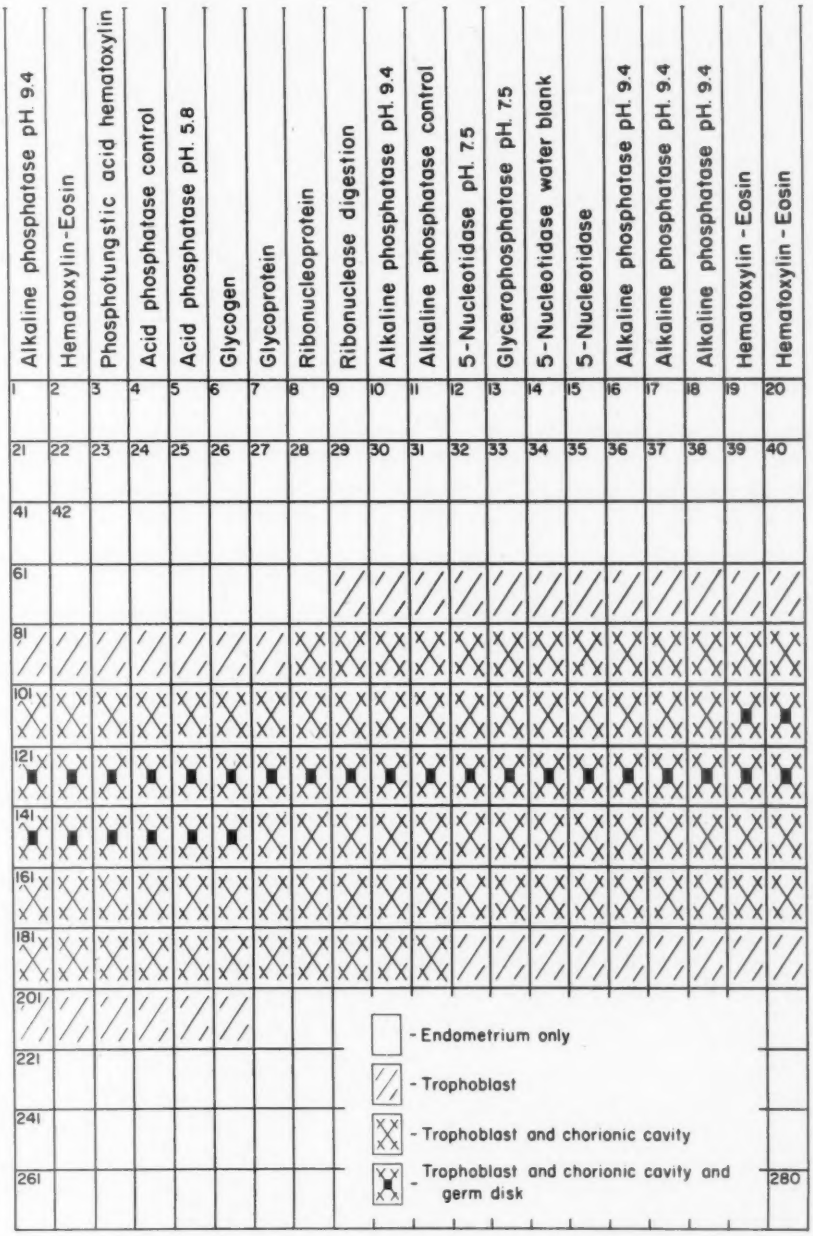

Fig. 1.—Distribution of serial sections for stains or enzyme reactions.

blast is, in general, distally or peripherally located and is the main contact of the ovum with maternal tissue. The cytotrophoblast is more proximally or centrally situated, forming the chorionic membrane and early primordial villi whose tips are distally in contact with endometrial stroma (Figs. 17, 20, 20*C*, 28, and 28*C*).

The trophoblast at the implantation pole is well developed but poorly so at the abembryonic pole. This is a function of the depth of implantation, which is subject to some variation at this time of gestation.[2] Thus the abembryonic pole of the ovum is covered only by a thin layer of maternal epithelium of questionable viability, together with a coagulum composed of fibrin, cellular debris, and leukocytes (Peters' *Schlusscoagulum*).

Histochemical Features of the Ovum and Implantation Site

Adenosine-5-Phosphatase (Figs. 2, 18, and 19).—This specific phosphatase hydrolyzes the nucleotide to a nucleoside.

In the ovum this enzyme is confined to the cytoplasm of the most active of the distally situated syncytiotrophoblast lying in contact with endometrial stroma and glands (Figs. 18 and 19). The enzymatic activity in the stroma immediately surrounding the ovum is strikingly negative (Fig. 2) although prominent within the stroma and the glands beyond this negative zone.

Alkaline Phosphatase (Figs. 4, 12, and 14-17).—This nonspecific phosphatase is active in the alkaline range. The enzyme is present in all of the syncytiotrophoblast, more particularly the brush border, both layers of the germ disc, and in the endothelium of the endometrial spiral arterioles.

Acid Phosphatase (Fig. 5).—This nonspecific phosphatase, active in the acid range, is strikingly absent from the ovum but is present in the *Schlusscoagulum* of the healing endometrial defect at the abembryonic pole and in the endometrial glands.

Glycogen (Figs. 6, 10, 20, 20C, 22, 22C, 24, 24C, 26, 26C, 28, 28C, 30, and 30C).—This polysaccharide is abundant in the ovum and surrounding endometrium. It is most prominent in the endoderm and cytotrophoblast, with lesser amounts in the ectoderm, chorionic mesoblast, yolk sac, and amnion. Although present in the syncytiotrophoblast, it is in the form of prominent, rounded or ovoid clumps rather than as isolated angular masses or aggregates seen elsewhere. Within the endometrium it is confined to the cytoplasm of the stromal and glandular cells.

Glycoprotein (Figs. 7, 11, 13, 21, 23, 25, 27, 29, and 31).—This complex of carbohydrate and protein is found within both the ovum and the endometrium. In the former it is found in the condensed magma or chorionic mesoblast on the

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Figs. 2-7.—The sections, Figs. 2-7, are at 45 diameters of magnification, except Fig. 2 which is 40, and pass through or near the maximum diameters of the trophoblastic shell and the germ disc. The pictures are oriented so that the ectoderm of the germ disc is dorsal or uppermost.

Fig. 2.—Adenosine-5-phosphatase (pH 7.5) is confined to the actively invading portions of the syncytiotrophoblast and the endometrium at some distance from the ovum. Note especially the absence of the enzyme from the endometrium immediately around the ovum. Greater detail is seen in Figs. 18 and 19.

Fig. 3.—Glycerophosphatase activity at this pH 7.5 is negative, thus indicating specificity seen in Fig. 2. Note absence of enzyme from ovum and endometrium. The dark foci in individual cells are nuclei counterstained with hematoxylin.

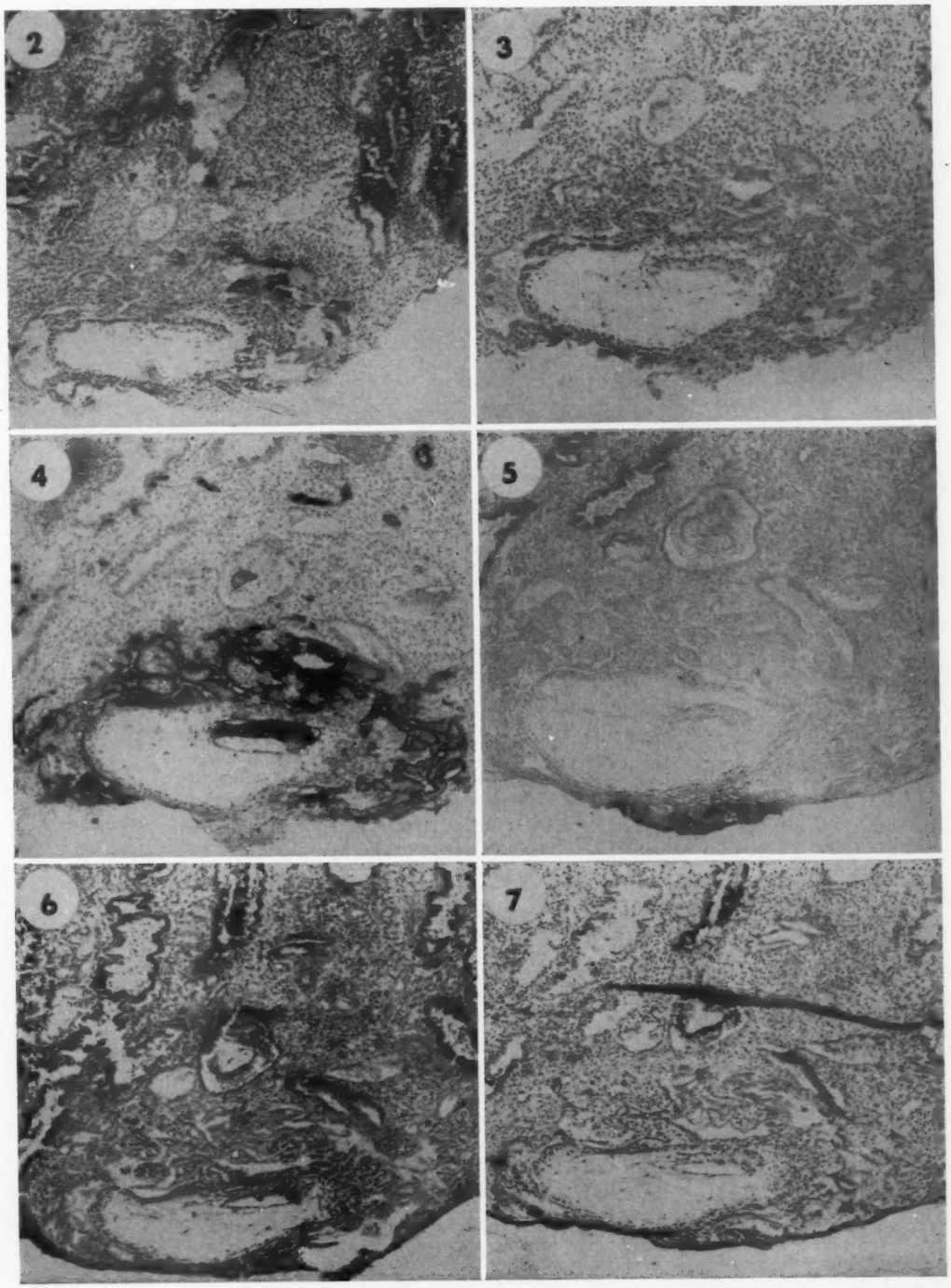
Fig. 4.—Alkaline phosphatase (pH 9.4) is concentrated in the syncytiotrophoblast, more particularly in the brush border, the germ disc, and in the endothelium of the endometrial arterioles and capillaries. For detail see Figs. 12 (germ disc) and 14 (trophoblast).

Fig. 5.—Acid phosphatase (pH 5.8) is absent from ovum but relatively prominent in *Schlusscoagulum* with lesser amounts in endometrial glandular epithelium.

Fig. 6.—PAS, undigested, to show presence of glycogen and glycoprotein. Compare with Fig. 7 for evidence that glycogen is heavily concentrated in germ disc, cytotrophoblast, glandular epithelium, and to a lesser extent in endometrial stroma. For greater detail of germ disc, see Fig. 10, and of trophoblast and implantation site, Figs. 20, 20C, 22, 22C, 24, 24C, 26, 26C, 28, 28C, 30, and 30C.

Fig. 7.—PAS digested to show glycoprotein after saliva digestion of glycogen. Note presence of concentrated glycoprotein in blocked gland beneath ovum and its absence in the unblocked glands elsewhere. Higher-power details of germ disc may be seen in Figs. 11 and 13 and of trophoblast in Figs. 21, 23, 25, 27, 29, and 31.

surface of the chorionic cytotrophoblast near the germ disc. Its concentration is presumably an artifact of fixation. It is also found in similar concentration within the yolk sac cavity on the surface of the endoderm (Figs. 11 and 13). It also forms a basement membrane between the layers of ecto- and endoderm (Fig.



Figs. 2-7.—*For legends see opposite page.*

13.) Within the intervillous space PAS-positive material is present in a mixture of maternal fibrin as shown by PTAH stain in Fig. 9 and the contents of an eroded endometrial gland as seen in Figs. 7 and 25. The syncytiotrophoblast is faintly positive for this stain. Within the endometrium glycoprotein is prominent in the gland lumina and in the apical tips of glandular cells. It is less prominent in the intercellular spaces of the stroma; and occasional stromal cells contain the material within their cytoplasm. Early decidual cells about the ovum have a PAS-positive border, the compressed reticulum known to surround the mature decidual cell.

Ribonucleic Acid (RNA) (Fig. 8).—This diffuse cytoplasmic basophilic substance—believed to be related to protein synthesis—is commonly found in rapidly growing tissues. In this specimen it is most prominent in the cytoplasm of the syncytiotrophoblast except for the brush border but present in lesser amounts in chorionic mesoblast and cytotrophoblast. Within the ectoderm it is readily visible as fine-to-coarse granules but is scant in the endoderm. Within the endometrium, RNA is confined to the basal third, in keeping with the observation in normal endometrium.[18] These authors showed this substance to be prominent in the proliferative phases of the menstrual cycle but absent in the secretory phase except for the basal portions.

Histochemical Details of Apparent Physiological Significance

Trophoblast.—The presence of adenosine-5-phosphatase in the actively invading syncytiotrophoblast and its absence in the immediately surrounding endometrium but its presence elsewhere in the implantation site is striking (Fig. 2). The presence of the enzyme in the actively invasive syncytiotrophoblast suggests that it may be concerned with the digestion and ingestion of stroma (Fig. 18) and glands (Fig. 19). At the junction of syncytiotrophoblast with endometrial glandular epithelium and stroma the enzyme also appears in the maternal cells. This may be a reactivation of the enzyme intrinsic in these maternal cells or may be evidence of invasion by tongues of syncytium that carry the enzyme into these maternal cells. Adenosine-5-phosphatase activity is present within syncytium throughout pregnancy especially in the brush border.[10]

In contrast, syncytiotrophoblastic alkaline phosphatase is much more concentrated at this stage in the brush border than in the cytoplasm of the syncytiotrophoblast (Figs. 4, 14-17). Since the brush border lines the intervillous space—and its precursors, the lacunae—the concentration of the enzyme here

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Fig. 8.—RNA. Note generalized basophilia of cytoplasm of germ disc, cytotrophoblast, syncytiotrophoblast, and endometrial glands and stroma. (×45.)

Fig. 9.—PTAH stain to delineate fibrin within the intervillous space, lower left, and within the blocked uterine gland above and to the right, which also communicates with the intervillous space, a phenomenon normal at this stage of implantation. Pure blood, without fibrin, is seen within the intervillous space in the lower right. (×45.)

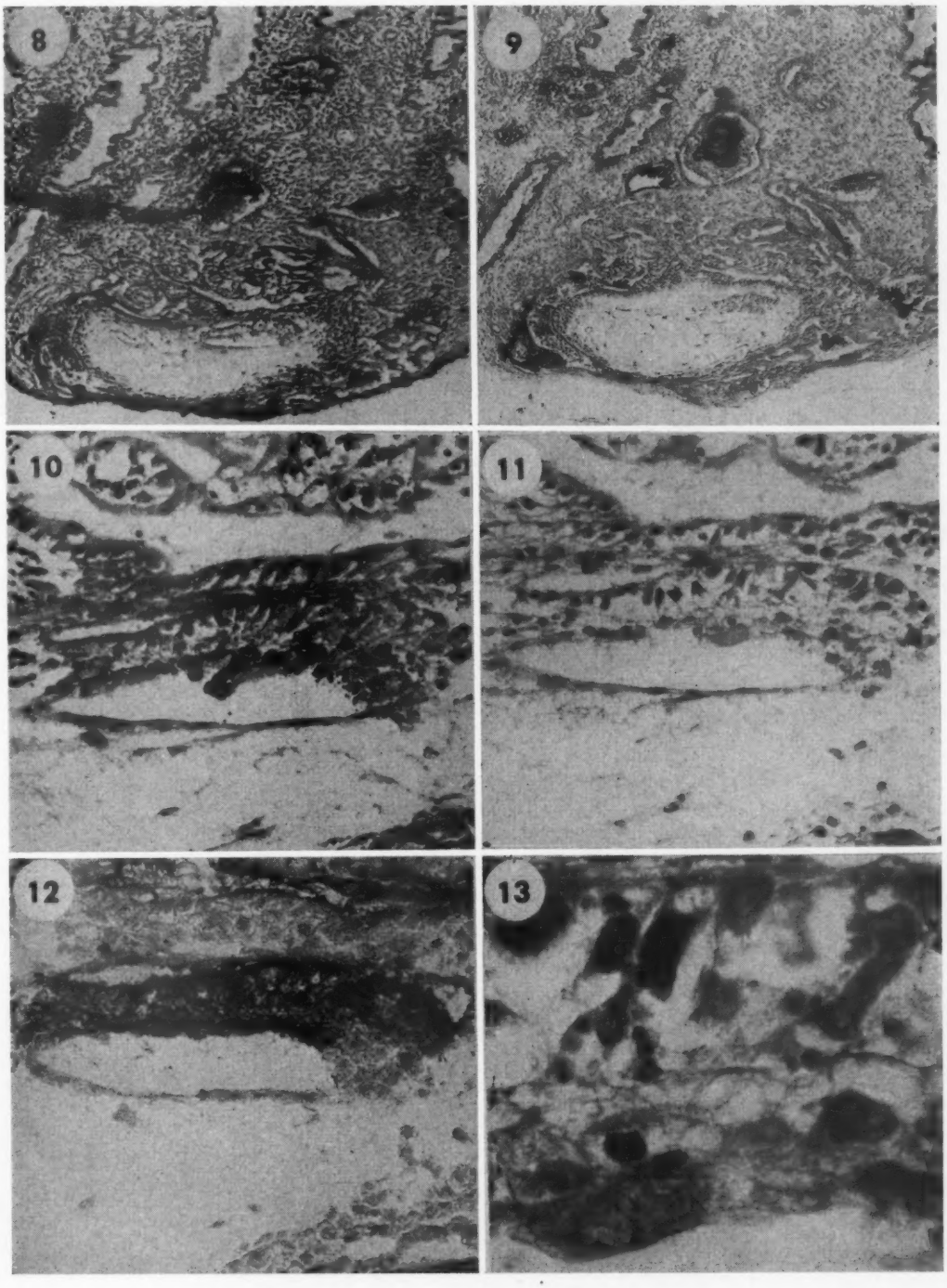
Fig. 10.—PAS, undigested, of germ disc and adjacent trophoblast. All of this darkly staining material in the endoderm, ectoderm, mesoblast, yolk sac, amnion (collapsed between ectoderm and trophoblast) and cytotrophoblast is glycogen as shown by its absence in the digested contiguous section seen in Fig. 11. (×200.)

Fig. 11.—PAS, digested, to show minimal amounts of glycoprotein in the mesoblast, germ disc with its membranes, and trophoblast. See Fig. 13 for higher-power detail of the central portion of the germ disc. (×200.)

Fig. 12.—Alkaline phosphatase (pH 9.4) is concentrated in the endoderm, ectoderm, and syncytiotrophoblast, particularly in its brush border. (×175.)

Fig. 13.—PAS, digested, to show detail of endo- and ectoderm. The glycogen has been digested. Note fine but distinct basement membrane, running horizontally through the center of the picture, separating ectoderm above from endoderm below. Within the latter are two types of cells: the numerous small, cuboidal, glycogen-filled variety interpreted as endoderm, one of which is in prophase, lower left, and the occasional large, round, glycogen-filled variety interpreted as a primordial germ cell lying against the basement membrane on the right. Note dark amorphous mass of glycoprotein, lower left, which is probably an artifactitious concentration. Note two clusters of basophilic "cytoid" bodies within the glycogen-filled but now empty columnar cells of the ectoderm. (×900.)

suggests that it may play a role in transfer of materials across the placental barrier. It is perhaps of some interest in this regard that the spiral arteriolar and capillary endothelium contain large amounts of this enzyme whereas the venous endothelium does not (Figs. 4, 14-17).



Figs. 8-13.—*For legends see opposite page.*

Glycogen and glycoprotein stains (periodic acid-Schiff, undigested and digested, respectively) reveal the role played by the cytotrophoblast in the production of placental site giant cells and the ability of the syncytiotrophoblast to ingest maternal tissue. Formerly it was thought that the placental site giant cells were of syncytiotrophoblastic origin but this specimen shows several good examples (Figs. 20, 20*C*, 22, 22*C*, 28, and 28*C*) of isolated glycogen-containing cells of cytotrophoblastic origin lying within the endometrial stroma. McKay and associates[10] described this in the placental sites of older pregnancies and noted it with respect to this specimen.

The ingestion of endometrial glandular epithelium is particularly well seen in Figs. 24 and 24*C*. The shadowy outlines of maternal nuclei undergoing necrosis are well seen in the contiguous section PAS digested as shown in Fig. 25. These two sections illustrate well that the invasive and/or erosive action of syncytiotrophoblast also includes actual phagocytosis or ingestion of maternal tissue. This was postulated in earlier observations of two of us[8] on H. and E. stained sections of the 11- and 12-day specimens but these special stains for glycogen and glycoprotein on the present specimen clarify this process. The ingestion of endometrial stromal cells is well illustrated in Figs. 26 and 26*C* and 28 and 28*C*. The syncytiotrophoblast appears to merge with (Figs. 26 and 26*C*) or actually surround and engulf isolated stromal cells (Figs. 28 and 28*C*).

Perhaps the most strikingly revealed activity of the syncytiotrophoblast with respect to its glycogen is the ingestion of this maternally derived material and its agglomeration into large rounded or oval masses (Figs. 24, 24*C*, 30, and 30*C*). We have chosen to designate this as the "lollipop" phenomenon because the large masses of glycogen suggest a lollipop during its consumption by a small child. Certainly the syncytiotrophoblastic glycogen is not in the fine-to-coarse angular aggregations (probably fixation artifacts) seen in the cytotrophoblast or the germ disc. Moreover, the presence of these large rounded or ovoid masses can be demonstrated at the site of ingested glandular epithelium which is particularly rich in this material (compare Figs. 24 and 24*C*, undigested, with Fig. 25, digested). The syncytiotrophoblast loses this phagocytic property after the

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Fig. 14.—Alkaline phosphatase (pH 9.4) in the actively invading syncytiotrophoblast is concentrated mainly in the brush border but small amounts of the enzyme are present in the cytoplasm. Note communication of the intervillous space, containing maternal blood, with capillary whose endothelium also contains the enzyme. Note absence of enzyme in endometrial stroma (right) except for endothelium of arterioles and capillaries. The lower-power view of the entire ovum and surrounding endometrium is seen in Fig. 4, the area in question being at the lower right. (×200.)

Fig. 15.—Alkaline phosphatase (pH 9.4) reacted section, not including the germ disc, shows general distribution of enzyme in syncytiotrophoblast and maternal arterioles and capillaries. The area at lower right is seen in higher detail in Figs. 16 and 17. (×45.)

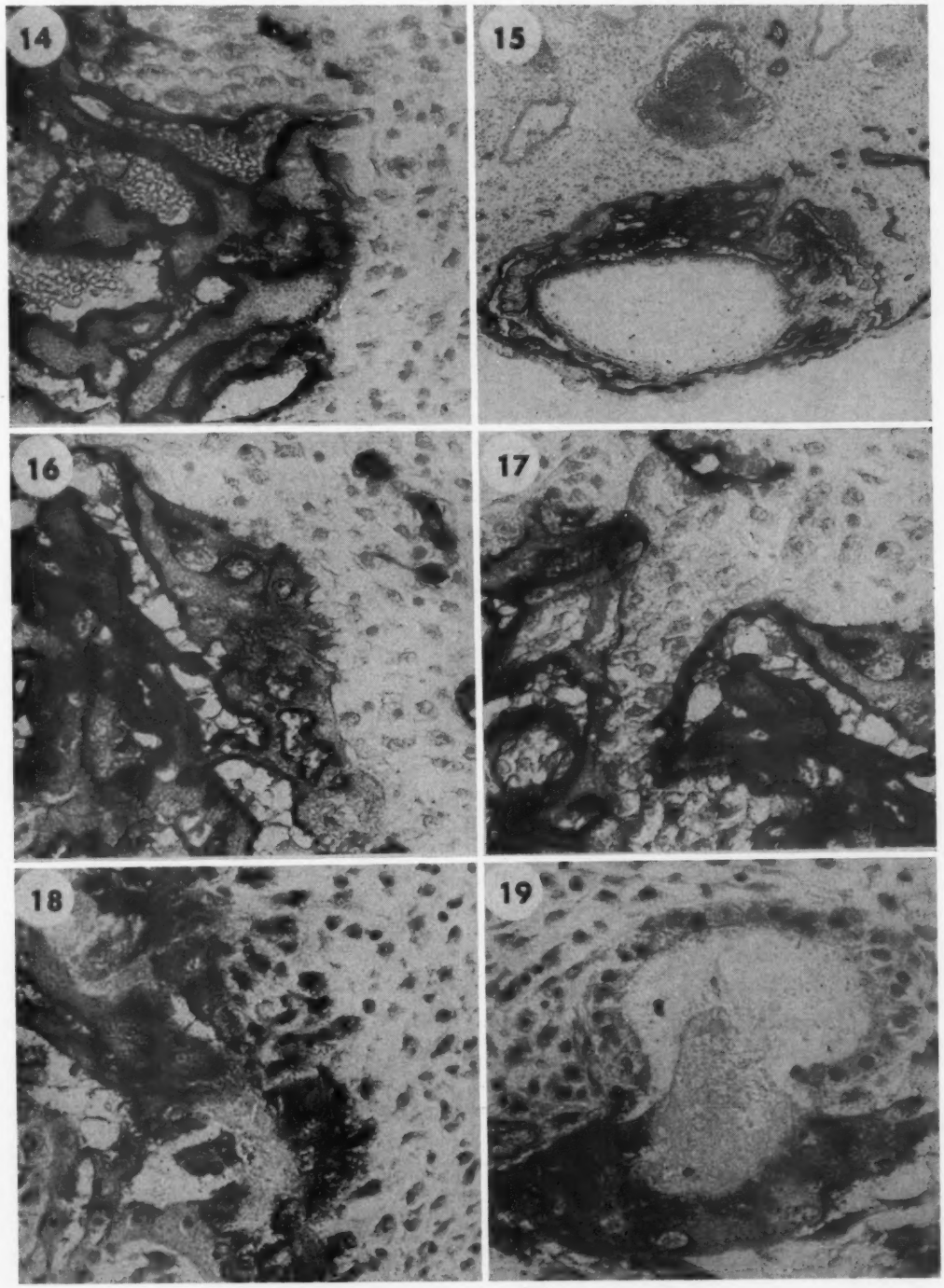
Fig. 16.—Alkaline phosphatase (pH 9.4) is diffusely distributed throughout the cytoplasm of the actively invading syncytiotrophoblast but is seen in greater concentration in the brush border lining lacunae which, after coalescence, form the intervillous space. Note streamers of syncytiotrophoblast imperceptibly merging with endometrial stroma which is being ingested and digested by the invading trophoblast. For orientation of this field see lower right of Fig. 15. (×200.)

Fig. 17.—Alkaline phosphatase (pH 9.4) to show detail of junction of cytotrophoblast of primordial villus with endometrial stroma. Note that both tissues are devoid of enzyme which is, however, prominent in maternal capillary at upper right and in syncytiotrophoblastic brush border. For orientation see right lower portion of Fig. 15. (×200.)

Fig. 18.—Adenosine-5-phosphatase (pH 7.5), in higher detail, shows concentration of enzyme in cytoplasm of actively invading syncytiotrophoblast. Note streamers of trophoblast merging with endometrial stromal cells—also containing the enzyme—although the latter is absent in the maternal tissue just beyond the ovular maternal junction. This area is oriented by consulting right lower portion of Fig. 2 and is nearly contiguous with comparable areas in Figs. 15 and 16 (alkaline phosphatase, pH 9.4). Compare Figs. 16 and 17 with Figs. 18 and 19 to show different concentration of these two phosphatases in actively invading syncytiotrophoblast. (×200.)

Fig. 19.—Adenosine-5-phosphatase is concentrated within the cytoplasm of syncytiotrophoblast which is actively eroding, ingesting, and digesting glandular epithelium. The remnant of the partially eroded gland is seen above; its contents lying in contact with the cytoplasm of the trophoblast. Remnants of gland cell nuclei are seen within syncytiotrophoblast at lower left. Note presence of enzyme in endometrial stromal cells immediately adjacent to trophoblast at lower left but absent elsewhere. (×200.)

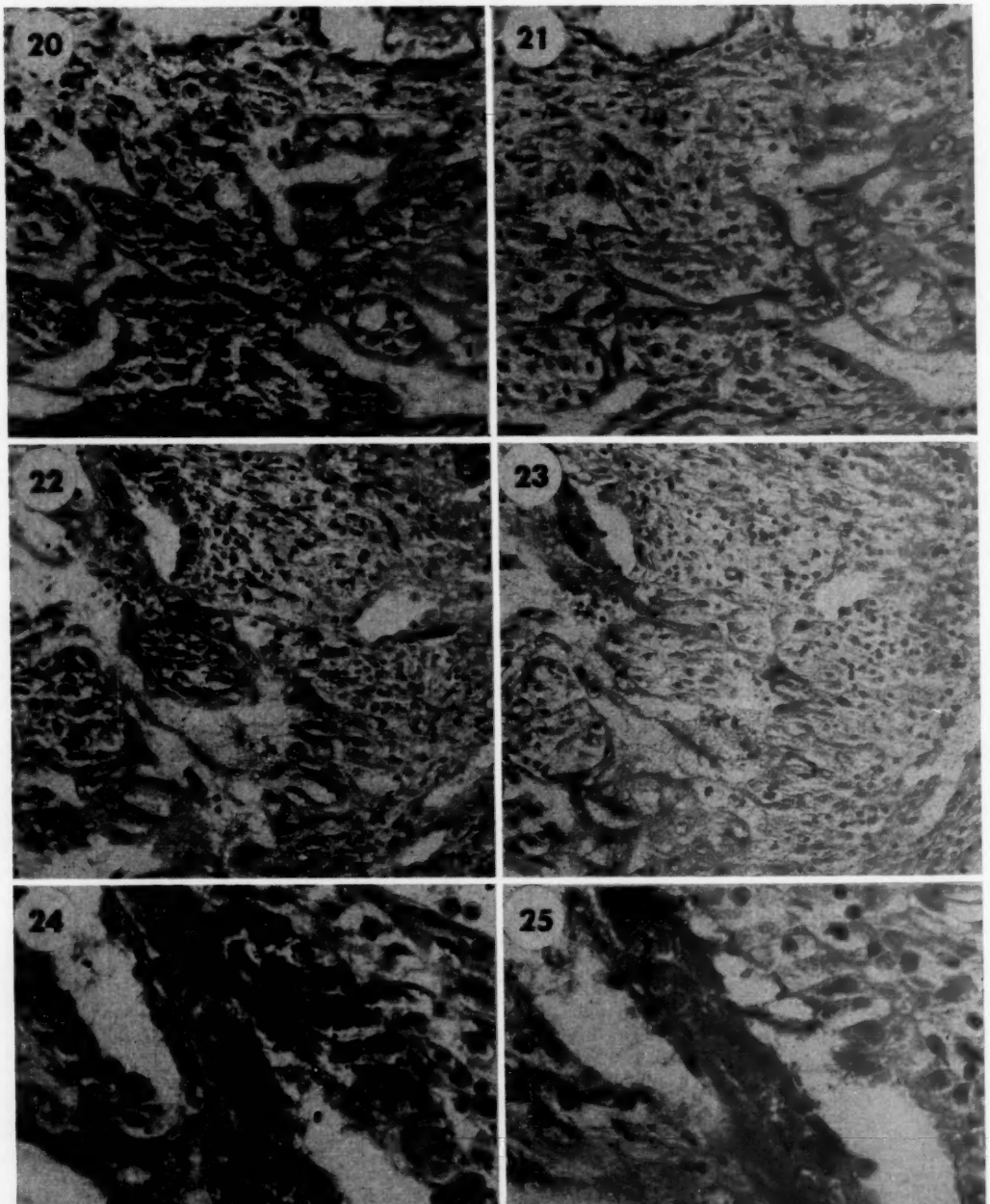
cytotrophoblastic "floor" or plate of the immature placenta is formed when primordial villi have simple branches, the age of the gestation being about 15-to-16 days. The concentration of glycogen within the syncytiotrophoblastic cytoplasm suggests that this tissue uses the substance for food. The cytotrophoblast



Figs. 14-19.—*For legends see opposite page.*

and the germ disc are heavily laden with glycogen and the relatively even distribution of this substance within these structures suggests its intracellular synthesis.

The large concentrations of cytotrophoblastic glycogen in the primitive villi may be a carbohydrate storage mechanism and give evidence of the site of



Figs. 20-25.—*For legends see opposite page.*

glucose regulation for the structures within the chorionic cavity. In this specimen, since there is no fetal circulation, the embryo derives its nutrition from material in the chorionic and thence from the amniotic and yolk sac cavities. Villee[19] has studied the regulation of blood glucose in the human fetus. His data show "that the liver of the human fetus is unable to assume its roles of storing glycogen and regulating the glucose concentration of the blood until after some 12 to 14 weeks of development and does not reach its full activity until after some 20 to 24 weeks of development or even later. Presumably the enzyme glucose-6-phosphatase, necessary for the secretion of glucose, does not develop in the liver cells as rapidly as certain other enzymes." He continues with respect to the placenta: "The experiments show that the placenta, early in gestation, does have the biochemical mechanisms necessary for the storage of glycogen and for the secretion of glucose. Late in gestation and at term the placenta has lost the ability to secrete glucose." The presence of glycogen in the villous cytotrophoblasts of this specimen may give a clue to the site of this glucose-secreting enzyme in the very early placenta. One of their derivations, the Langhans layer, present in the placental barrier through approximately the sixteenth week of gestation, may function as the glucose-regulating cells of the early placenta. The histochemical demonstration of glucose-6-phosphatase was not attempted as fresh frozen sections are required to maintain the activity of this enzyme.[20] McKay and co-workers[13] have discussed the role of the early yolk sac in the 5 to 7 mm. stages as a structure that supplies glucose to the embryonic circulation before the fetal liver is capable of regulating the glucose level.

The amnion and yolk sac, of trophoblastic origin but now resembling mesoblast, have the histochemical properties of the latter tissue. Their cells have small amounts of RNA and glycogen and only minimal amounts of alkaline phosphatase, doubtless sufficient for their own metabolic needs and for the minimal function these primitive membranes may possess at this stage of development.

Germ Disc.—Within the germ disc glycogen is mostly concentrated in the endoderm although lesser amounts occur in the ectoderm. Within the endoderm and delineated from ectoderm by a distinct basement membrane are occasional very large cells with a prominent cell membrane, whose cytoplasm is stuffed with glycogen (Figs. 10, 11, and 13). The size and general morphology of these occasional cells—located near the edge of the germ disc—suggest that they are

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Figs. 20-25.—These are all stained by the PAS method for glycogen and glycoprotein. (See Figs. 20*C*, 22*C*, and 24*C* for colored reproduction.) Those on the left show glycogen plus glycoprotein whereas contiguous sections on the right, because of saliva digestion, show only glycoprotein. Thus the very dark areas within the cytoplasm of all cells, ovular as well as maternal, represent glycogen. For orientation, see right and middle regions of Figs. 6 and 7.

Fig. 20.—PAS, undigested, shows great concentration of glycogen in cytotrophoblast (lower four-fifths of picture) and less in cytoplasm of endometrial stroma and glands (top). Note lesser amounts of glycogen in cytoplasm of syncytiotrophoblast at lower right. (×175.)

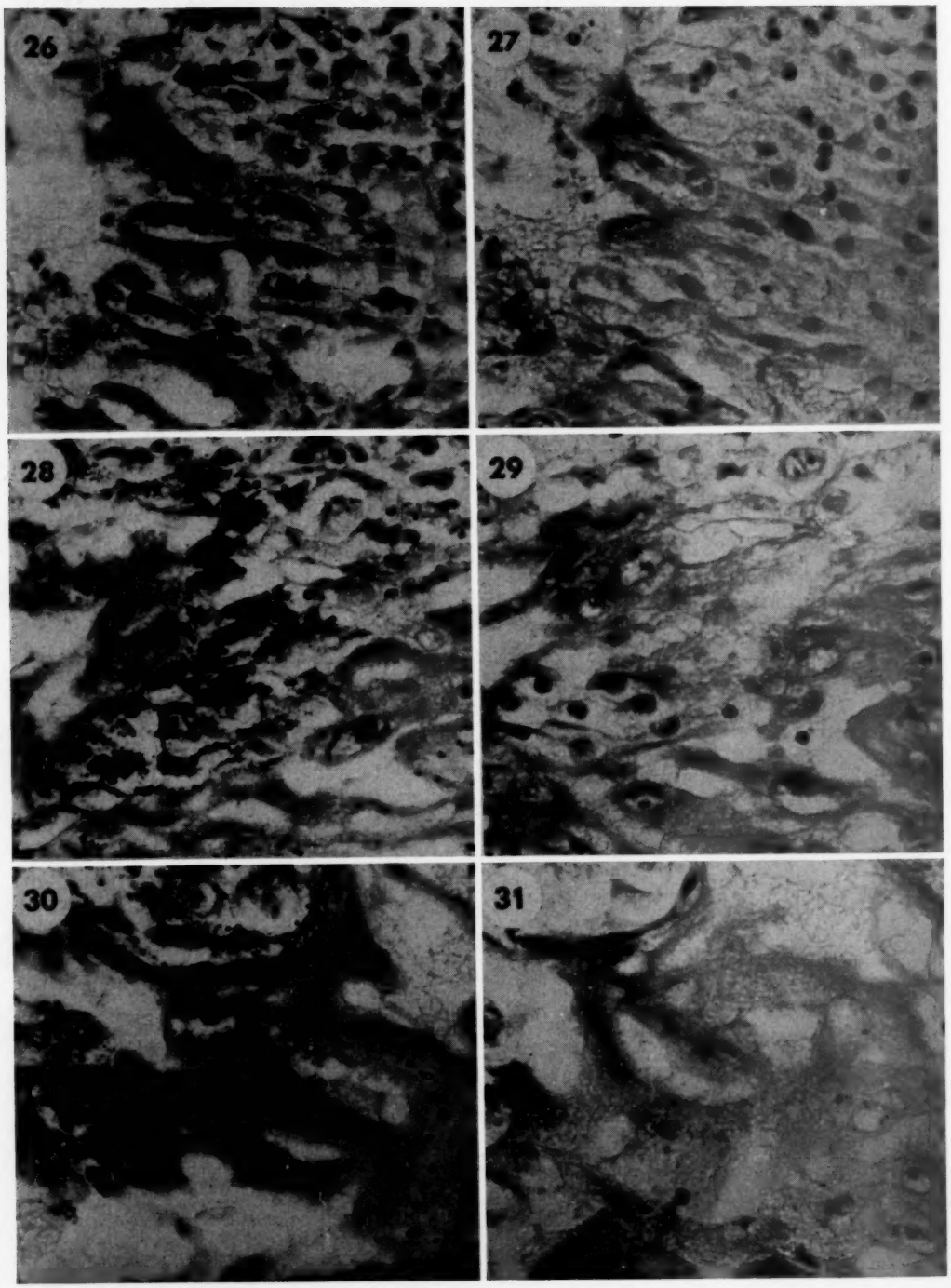
Fig. 21.—PAS, digested, to show absence of glycogen but small amounts of glycoprotein in cytoplasm of all types of cells. Compare with Fig. 20. (×175.)

Fig. 22.—PAS, undigested, shows trophoblast in left lower half and endometrium in right upper half of picture. Note glycogen-containing cell—of cytotrophoblastic origin—streaming out into the endometrial stroma beneath the large venous sinusoid. The communication between gland lumen and intervillous space is well shown in upper left. The syncytiotrophoblast is ingesting glandular epithelium and contents, inspissated glycoprotein, and questionable amounts of glycogen—a phenomenon well seen even at this power but in greater detail in Fig. 24. It may also be seen in the adenosine-5-phosphatase preparation in Fig. 19. (×100.)

Fig. 23.—PAS, digested, shows that contents of gland being ingested by syncytiotrophoblast and being incorporated into the intervillous space are indeed composed largely of glycoprotein and contain little if any glycogen. Greater detail is seen in Fig. 25. (×100.)

Fig. 24.—PAS, undigested, shows communication of eroded gland (seen in upper left of Fig. 22) with intervillous space and the ingestion with subsequent digestion of glandular epithelium by syncytiotrophoblast. Note abundance of glycogen in glandular epithelium but especially the large rounded aggregates of glycogen, derived from epithelium, within this actively invasive and destructive syncytiotrophoblast. The shadowy remnants of glandular nuclei undergoing digestion are seen intermingled among the glycogen masses. Such cellular remains are better appreciated in Fig. 25. For orientation see Fig. 22. (×400.)

Fig. 25.—PAS, digested, shows that the glandular contents are largely, if not entirely, composed of glycoprotein. Note in lower center the remnants of ingested glandular epithelium in the form of nuclei undergoing pyknosis and karyolysis. For orientation see Fig. 23. (×400.)



Figs. 26-31.—These are PAS stained; those on the left are undigested (see Figs. 26*C*, 28*C*, and 30*C* for colored reproduction) and represent contiguous sections with those on the right which are digested. The dark cytoplasmic masses in pictures on the left thus represent glycogen. For orientation, see right lower regions of Figs. 6 and 7.

For individual legends see page 1039.

Fig. 20*C*.

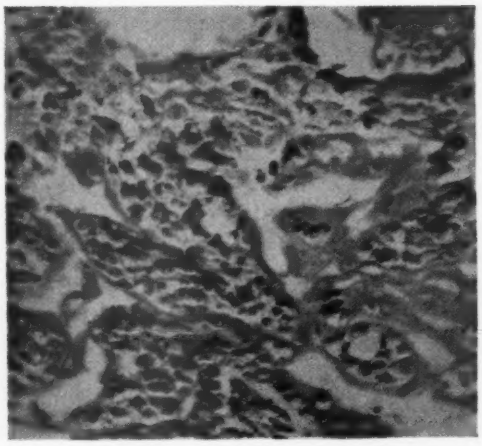


Fig. 26*C*.

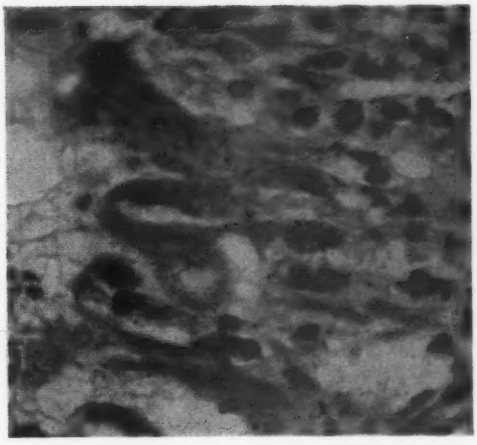


Fig. 22*C*.

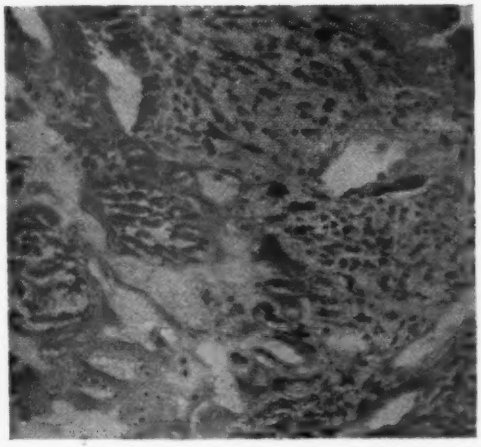


Fig. 28*C*.

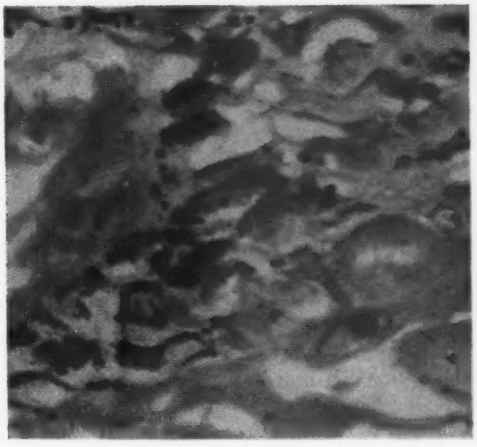


Fig. 24*C*.

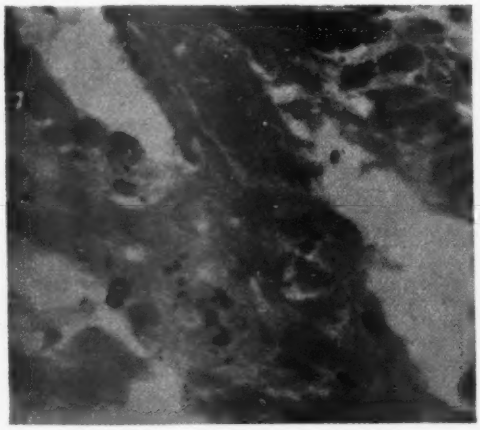
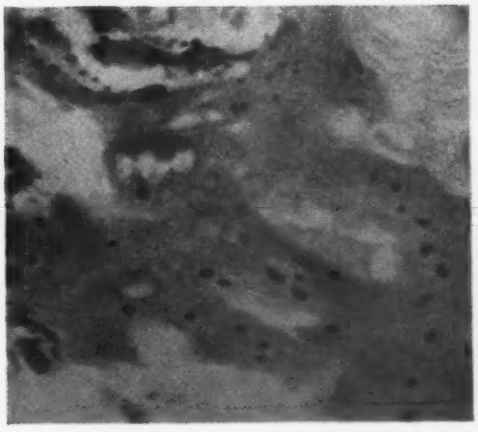


Fig. 30*C*.



Figs. 20*C*, 22*C*, 24*C*, 26*C*, 28*C*, and 30*C* are colored reproductions of the corresponding black and white illustrations of PAS glycogen seen in Figs. 20, 22, 24, 26, 28, and 30. Please refer to those legends for descriptions of these figures.

primordial germ cells. We have seen suggestive evidence of such a cell in the 4½ day 107 cell blastocyst (Carnegie No. 8663, Fig. 6[2]), whose embryo contains 8 cells. Thus the presence of one or more primordial germ cells at 13 days is reasonable since Witschi[21] described approximately 40 of them in the caudal end of the yolk sac endoderm of the 13 somite embryo estimated by Streeter[22] to be 24± days' ovulation age. McKay and collaborators[23] studied histochemically migration of the primordial germ cell from the gut endoderm of the 5 mm. embryo (28 days' developmental age) to the definitive gonads of the 23 mm. male embryo (42 days') and the 35 mm. female embryo (56 days' developmental age).

Within a few cells of the ectoderm are several clusters of small, rounded, basophilic masses which resemble tiny cells (Fig. 13). Their basophilic centers suggest chromatin surrounded by a tiny halo of cytoplasm. Since they occur within normal-appearing cells and since they are evident in other normal human embryos of this stage[2] and in the macaque monkey embryos[24] of comparable development, they are undoubtedly normal. In this specimen they are near the center of the germ disc and concentrated nearest the endoderm. The function of such "cytoid" bodies is unknown. Perhaps they play a part in the induction of primitive streak formation with its sequelae of mesoderm formation and axial differentiation.

Although morphological differentiation of the germ disc is almost imperceptible during Horizons V and VI, its high alkaline phosphatase activity is characteristic of the initiation of rapid differentiation in embryological tissue,[25] its ribonucleoprotein content is indicative of protein synthesis, and its glycogen content is evidence of carbohydrate reserve for energy. Within a few hours the germ disc will begin its rapid morphological differentiation in which these histochemically demonstrated constituents undoubtedly play a part.

Summary and Conclusions

1. A 13 day early villous human ovum of Streeter's Horizon VI, serially sectioned and reacted histochemically or stained for alkaline phosphatase, acid phosphatase, adenosine-5-phosphatase, glycogen, glycoprotein, and RNA has been described.

2. The bilaminar germ disc is rich in alkaline phosphatase and glycogen with moderate amounts of RNA and small amounts of glycoprotein. The latter is most prominent as a basement membrane between ecto- and endoderm.

Fig. 26.—PAS, undigested, shows incorporation or ingestion of endometrial stromal cells on the right by syncytiotrophoblast on the left. The irregular space on the left is the intervillous space, whereas a maternal venous sinusoid is seen in the lower right. (×400.)

Fig. 27.—PAS, digested, shows dark endometrial stromal cell, with poor nuclear detail, merging with syncytiotrophoblast. (×400.)

Fig. 28.—PAS, undigested, shows junction of glycogen-rich cytotrophoblast of primordial villus merging with endometrial stroma. Note streaming of 3 cytotrophoblastic cells into endometrial stroma at upper middle, a source of placental site giant cells. Note further the encirclement of single endometrial stromal cell, at extreme right middle, by invading syncytiotrophoblast, one of the mechanisms by which the trophoblast ingests maternal tissue. (×400.)

Fig. 29.—PAS, digested, shows small amounts of glycoprotein in syncytiotrophoblast but is excellent to show shadowy or pyknotic remnants of ingested endometrial stromal nuclei. (×400.)

Fig. 30.—PAS, undigested, from equatorial region of ovum, shows large smooth masses or aggregates of glycogen within the cytoplasm of the syncytiotrophoblast. These masses are interpreted as undergoing digestion and were derived from ingested endometrial stromal cells—the so-called "lollipop" phenomenon. Contrast these large aggregates of ingested glycogen in the syncytiotrophoblast with those of presumably endogenous nature found in cytoplasm of cytotrophoblast in left and left upper portion of photograph. (×400.)

Fig. 31.—PAS, digested, shows absence of glycogen in section contiguous to that shown in Fig. 30. (×400.)

3. The primitive amnion and yolk sac, derived from cytotrophoblast, contain moderate amounts of glycogen as does the chorionic mesoblast, also derived from cytotrophoblast.

4. The cytotrophoblast is rich in endogenous glycogen and is presumably a source of placental glucose known to be formed early in pregnancy.

5. The syncytiotrophoblast is rich in alkaline phosphatase, especially in the brush border, and rich in adenosine-5-phosphatase, most prominent within the cytoplasm. Ribonucleoprotein is present in this layer except at the brush border.

6. The syncytiotrophoblast contains prominent aggregates of glycogen and cellular remnants undergoing digestion, derived from ingested endometrial glandular and stromal cells.

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Discussion

DR. JOHN I. BREWER, Chicago, Ill.—It is an honor and a pleasure for all of us to be here today to hear and see the presentation of processes of living growth and development in a beautiful young ovum. Truly indicative of the advance in our knowledge of human development, achieved principally by the studies of such workers as Hertig, Rock, and their co-workers, Streeter, Heuser, Corner, Hartman, Bartelmez, and Carl Wilson, is the simple fact that Hertig and co-authors had the courage to fix a normal 13-day-old ovum in acetone and perform histochemical studies. Even so, I imagine Dr. Hertig acceded only after a gentle but persistent push by Dr. McKay and Eleanor Adams. To all these workers we express our gratitude for and pride in their real scientific accomplishments.

Nucleoproteins, which have been demonstrated in the rapidly growing fetal tissue by the authors, are vital for growth and development. These are composed of compound proteins

with nucleic acid. Ribose nucleic acid (RNA), the characteristic nucleic acid of the cytoplasm, is located principally in the mitochondria and microsomes. The exact composition of mitochondria is not precisely known but since the essential work of Bensley and Hoerr in 1932 it has become generally accepted that these structures are made up of protein, ribose nucleic acid, lipid including considerable phospholipid, enzymes, and at least one coenzyme.

Hertig and his co-authors have demonstrated in this young ovum that RNA was most prominent in the cytoplasm of the syncytiotrophoblast. In this tissue mitochondria are most prominent, as shown by Jones and Brewer[1] in 1935. Less RNA was found in the cytotrophoblast and similarly Jones and Brewer found fewer mitochondria in this particular tissue. These observations seem consistent and are probably significant.

While all is not known concerning phosphatase, it is accepted that it is vital for the formation of bone and the metabolism of carbohydrates, nucleotides, and phospholipids. The location of alkaline phosphatase in the syncytium, particularly the brush border, led the authors to suggest its probable role in the transfer of materials across the placental barrier, a very plausible contention since carbohydrates, ribose nucleic acid, and phospholipids are known to be present in this region.

The authors have pointed out that the glycogen in the syncytium is in large masses in contradistinction to the fine droplet form noted in the cytotrophoblast. We have observed this same phenomenon in the syncytial wandering cells invading the endometrium in a slightly older human ovum.

The origin of these wandering cells and the syncytium seems to be the cytotrophoblast.[2] Cytotrophoblastic cells also invade the endometrium in young stages of development but in slightly older stages these cells have assumed the characteristics of syncytial cells without brush borders. Brush borders are acquired only by those syncytial cells or cell masses that are in contact with maternal material in the intervillous or potential intervillous spaces.

These syncytial wandering cells, the cytotrophoblastic cells that assume the characteristics of syncytium, and the multinucleated syncytial cell masses phagocytize, break up, and reduce to granular material many maternal elements such as reticulum, erythrocytes, and lymphocytes.[3]

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DR. EDWARD C. HUGHES, Syracuse, N. Y.—The histochemical observations of a normal human fertilized ovum at the thirteenth day of its intrauterine life have been particularly interesting to us because it has emphasized the importance of the functional activities of the environment to the nutrition, development, and growth of the embryo. It also demonstrates the utilization of the substances which have been shown to be metabolized within the structures of the endometrium and which are produced there primarily for the nutrition of the embryo and its related structures. The preparation of the material and demonstration of the histochemical reactions which take place between the ovum and endometrium have been magnificently demonstrated and illustrate the dependence of one on the other.

It appears that the chorion is the important anatomic structure in this process of nutrition of the ovum. Its various cellular components are particularly devoted to specific phases of the metabolic process. Apparently the syncytial trophoblast or the outer clothing of the villi and the cytotrophoblast have different activities but are necessary to one another for the complete function. It is fascinating to realize that the endometrium furnishes the necessary nutritive substances, namely glucose, glycoproteins, while the chorion establishes an exchange of these materials by a system of enzymal and chemical reactions.

The presence of alkaline phosphatase in the brush border of the syncytium indicates that there are possibly several functions of these cells. The secretion of this enzyme may facilitate the absorption of the glucose fraction of the substrate, which is presented by the endometrium, by splitting off glucose-6-phosphate, or it may aid in the maintaining of the pH

at this decidual chorionic junction, or it may have some relationship to the metabolism of calcium which is also necessary at this location for implantation. The secretion of alkaline phosphatase by these cells continues throughout pregnancy and it is found in great abundance in the syncytial cells at term. This tends to indicate that the syncytial trophoblast maintains this function, namely, transfer of material from the decidua to the fetus throughout gestation.

The Langhans cells, on the other hand, seem to be the more active elements of the chorion and possess several physiologic functions. There is no question that their prime purpose is to store glucose as glycogen and serve as the embryonic liver during this stage of gestation. This is a function that lasts into midpregnancy. Their second purpose, however, which is probably just as important, is the secretion of chorionic gonadotrophin, a glycoprotein hormone. The glucose derived from the cytotrophoblast is an important ingredient of this hormone. This feature itself probably constitutes the very important function of these cells because it is necessary to have chorionic gonadotrophin secreted in order to maintain the corpus luteum with its secretion of estrogen and progesterone which establishes the nutritional relationship between the decidua and the chorion.

These same histochemical reactions have been noted in a 24-day-old human fertilized ovum, estimated from the conception date. The specimen was received in our laboratory, having been obtained by biopsy curette. It was stained histochemically, fixed in absolute alcohol, mounted in a paraffin block after preparation, sectioned at 8 μ. Sections were stained for glycogen, by the PAS method of McMannus. Alkaline phosphatase staining was accomplished by the use of a modification of the Gomori stain and a pH of 9.5. RNA and DNA staining was by the Pappenheim-Unna method. Nucleoproteins were reacted by the Feulgen method. The over-all dimension of the ovum in the block measured 5 by 6 mm. The amniotic sac was quite large and filled with a pink-staining fluid. There was some fragmentation of the yolk sac which apparently had been damaged at the taking of the specimen. However, the sac was filled with many fairly large granules which stained for glycogen. The cephalocaudad length of the embryo was 3 mm.

Alkaline phosphatase was found in abundance in the brush border of the syncytial trophoblast and was particularly positive in the terminal tips of the villi as they seemed to penetrate the decidua. It was our impression that the syncytium per se did not contain glycogen, although the large circumscribed accumulation of highly staining glycogen granules which Dr. Hertig has referred to as lollipops were noted free within the syncytial structure.

Ribonucleic acid was noted to be present in the cytoplasm of the syncytial trophoblastic cells while desoxynucleic acid was found in the nuclei of the cells. The significance of these findings cannot be explained at this time but probably indicates cell activity.

The striking feature of the entire specimen was a large amount of glycogen which was stored in the cytotrophoblast, the mesoblast of the villi, and the yolk sac. Many villi indicated the beginning formation of the angioblastic structures adjacent to and immediately underneath the cytotrophoblastic layer. It was noticeable that these cells did not contain glycogen or any other nutritive substance. Large trophoblastic cells penetrated the decidua and incorporated large granules of glycogen. This specimen further substantiated the fact that these cells, which are found in the substance of the endometrium, were probably cytotrophoblastic in origin. They were persistently found penetrating the decidua in other specimens of our collection until approximately 120 days of gestation.

The embryo seemed to store glycogen in the dorsal ectoderm and somites. It was also found in small amounts in the early tubules of the kidney and in cells which apparently made up the endoderm and the neurotube. Early primitive embryonic liver cells did not contain glycogen and this further substantiated the fact that the cytotrophoblast serves as the liver at this time.

Embryonic brain did not possess any glycogen-staining material, a feature which is maintained throughout gestation. Failure of the endometrium to furnish these nutritive substances or failure of the chorion and other structures to be able to synthesize them probably plays an important role in the causation of unsuccessful pregnancy, namely, abortion, congenital malformation, and perhaps premature labor.

DR. JOHN ROCK, Brookline, Mass.—There seems to be a common concept that the enzymes in the endometrium are directly essential to the growth and development of the early embryo. I think this may be so to a certain extent, but only for a very limited period.

The endometrium, it seems, is built up for one major purpose: to supply blood to the conceptus. The glands also have a certain function to perform. This they do probably during what we call the *proliferative phase* when the significant secretion takes place in the epithelium of the glands. Bartelmez has shown that this material is not too obvious in fixed and stained sections because the myometrium is contracting and constantly squeezing out the product. In the uterine cavity, it offers a medium in which the ovum, after entry at about the fourth day, will grow into a blastocyst. In some of the other animals, the ovum grows into the trophoblast and even matures the embryo while still free. In the human and in other primates, the embryo depends on blood.

Up to the time of imbedding, the blastocyst utilizes the product of the glands, so we should not be surprised to find in the endometrium various enzymes, and to find in the glandular secretion food material. At imbedment, the syncytium phagocytizes the stroma of the endometrium. I wonder whether it needs this for food material, or does it merely remove it so as to gain access to maternal blood? As soon as the conceptus gets maternal blood there develop within it the enzyme systems which will take out of the maternal blood what it needs; and for nourishment it would not seem likely to depend on the tissue of the endometrium.

To be sure, in Dr. Hertig's 13 day stage we will find within the syncytium stromal cells containing glycogen and enzymes. These cells have been phagocytized, which does not necessarily mean that this material was there for the purposes of the embryo. Was it there, perhaps, merely for the purposes of the endometrium, as a supporting matrix for the essential vascular system? Does the syncytium merely have to get rid of these cells? We find the ovum growing in other tissues that have not such a complex setup. Further study is necessary to discover the nature and locations of the enzymes in other parts. We know from Fawcett's study of mouse ova transplanted into the anterior chamber of the eye, that the conceptus will grow only as long as the maternal vascular system is intact.

One other point comes to mind. The lacunae within the syncytiotrophoblast contain maternal blood, and there is doubtless a slight degree of pressure within the lacunae, for, as new blood comes in, so some blood must go out. In one of our sections, we can see syncytium breaking into a gland, and we can see glandular secretion in contact with the syncytium. This does not necessarily mean to me that the syncytium is feeding on it. It just happens to be bathed in it. There is little or no pressure in the glands of the late progestational phase; the pressure is in the lacunae. How the glandular secretion would function against the pressure of the arterial system has to be answered. I suggest that we go very carefully toward interpreting the significance to the conceptus of endometrial food material apart from maternal blood. Our progestational phase of the endometrium with its enzymes does not a priori contain them as directly useful to the conceptus.

DR. HERTIG (Closing).—The clear-cut evidence that Dr. Brewer gave that the primitive cytotrophoblast does form syncytiotrophoblast intrigues me. I have wondered about this and this evidence satisfies me very well. Therefore, it appears that the giant cells of the placental site, whether they are derived from the synctiotrophoblast that invades the endometrium or from the cytotrophoblast that has invaded the endometrium, seem mainly to be of cytotrophoblastic origin.

I agree with Dr. Rock that we must go slowly on interpreting what the lollipops are doing in the syncytiotrophoblast. We know that children put lollipops in their mouths deliberately for food, whereas the syncytiotrophoblast may just be getting material out of the way so that the ovum may be implanted and gain access to the maternal blood supply.

ADENOMYOSIS: A REAPPRAISAL OF SYMPTOMATOLOGY*

RALPH C. BENSON, M.D., AND VINTON D. SNEEDEN, M.D.,
PORTLAND, ORE.

(From Emanuel Hospital and University of Oregon Medical School Hospitals and Clinics)

ADENOMYOSIS is infrequently diagnosed and its symptomatology is not clearly understood by many physicians. This is a curious state of affairs because this disorder is certainly not new. Rokitansky[1] first described the condition in 1860. Von Recklinghausen's[2] well-publicized article on the clinicopathological features was published in 1896. Cullen's[3] unusually complete and now famous 1908 monograph, as well as other more recent studies, [4, 5, 6] has also described the disease exceedingly well. Confusion has developed because of the unfortunate and illogical inclusion of adenomyosis with pelvic endometriosis, which it only occasionally accompanies.[7] The clinical findings are often altered by the frequent association of other pelvic abnormalities which are also capable of causing symptoms similar to those of adenomyosis.[8]

For half a century, the manifestations of adenomyosis have been stated to be: progressively severe menstrual bleeding, increasingly painful dysmenorrhea, and a gradually enlarging uterus.[3] This concept may be true, but we know of no adequate study which relates adenomyosis to the patient's complaints —*after* eliminating other associated disorders. Bayly and Yates[9] indicated that patients with adenomyosis often bleed from accompanying abnormalities, but only this phase of the problem was considered. It appears that the symptomatology has been largely based upon clinical assumptions and generalizations. Be this as it may, adenomyosis deserves far more consideration than it has received, not only because of its frequency, but also because of the degree of disability which it causes.

The purpose of this investigation is: (1) to ascertain the rate of occurrence, extent, and accuracy of diagnosis of adenomyosis in a private general hospital; (2) to demonstrate how adenomyosis and concomitant significant abnormalities affect the patient; and (3) to show adenomyosis as a clinical problem exclusive of coexisting disorders capable of producing similar symptoms. Such a reappraisal should aid in earlier and more accurate diagnosis, as well as better treatment of abnormal uterine bleeding and pelvic pain.

Material and Methods

Only cases with endometrium extending into the myometrium *more* than two standard low-power fields and associated with muscle changes were accepted

*Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

as examples of adenomyosis. Included in this study are 701 consecutive cases representing all instances of this entity treated at Emanuel Hospital, Portland, Oregon, from Jan. 1, 1950, through Dec. 31, 1957, in women of 50 years of age or under, still having menses. The youngest patient was 18 years of age. The average age was 40.9 years, with a notable increase in the number of women with adenomyosis after 40 years of age.

During the 7 year period, 2,536 total and subtotal abdominal hysterectomies and 740 total vaginal hysterectomies were performed at this institution on women in the age group 18 through 50 years. Seven hundred and one instances of adenomyosis were found in 3,276 uteri, an incidence of 21.4 per cent. The operators were specialists and generalists with gynecological surgical privileges on this otherwise open staff.

The weight of the uterus was the major criterion of its degree of enlargement. It was not considered enlarged when the weight was less than 100 grams. For our purposes, slight enlargement was present when the weight was from 100 to 150 grams, moderate enlargement from 150 to 200 grams, and marked enlargement when over 250 grams. The extent of the adenomyotic process was also estimated by the depth of penetration and the relative proportion of the uterus involved.

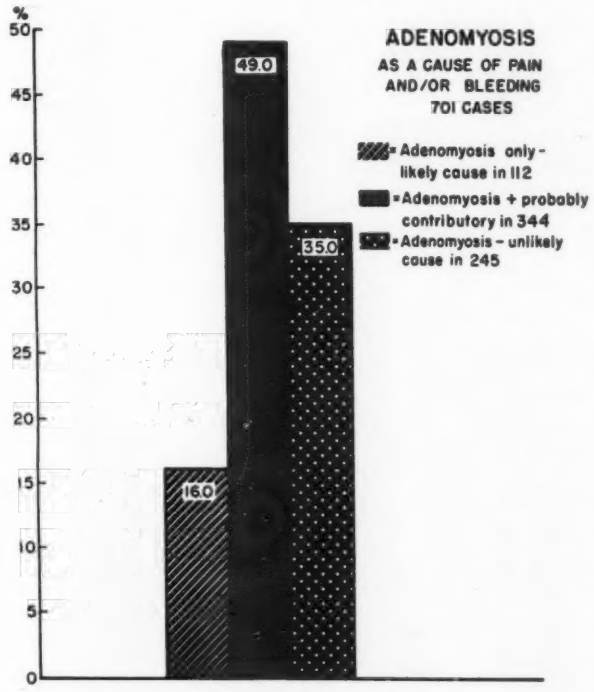

Fig. 1.

An attempt was then made to determine the probability of adenomyosis causing abnormal uterine bleeding. Admittedly, this can be only an opinion, and functional problems are minimized under these circumstances. Nevertheless, where nothing save significant adenomyosis was found to explain the gynecological complaints, adenomyosis was held to be the "likely" cause of the difficulty. There were 112 patients in this group (Fig. 1). When prominent benign endometrial involvement of the uterine wall was discovered in association with hypertension, myomas, or salpingitis, for example, the adenomyosis was

recorded as "contributory." We noted 344 patients in this category. In 245 cases, adenomyosis was discovered quite by accident—as in uteri removed for descensus alone. In such cases, adenomyosis was noted as "no cause" for menstrual aberration or distress.

Findings

Age Distribution.—A close similarity between the number of patients in each age breakdown can be seen in Fig. 2. The comparable nature of these groups is indicated by a difference of less than 3 years in the average age of the individuals in the three categories.

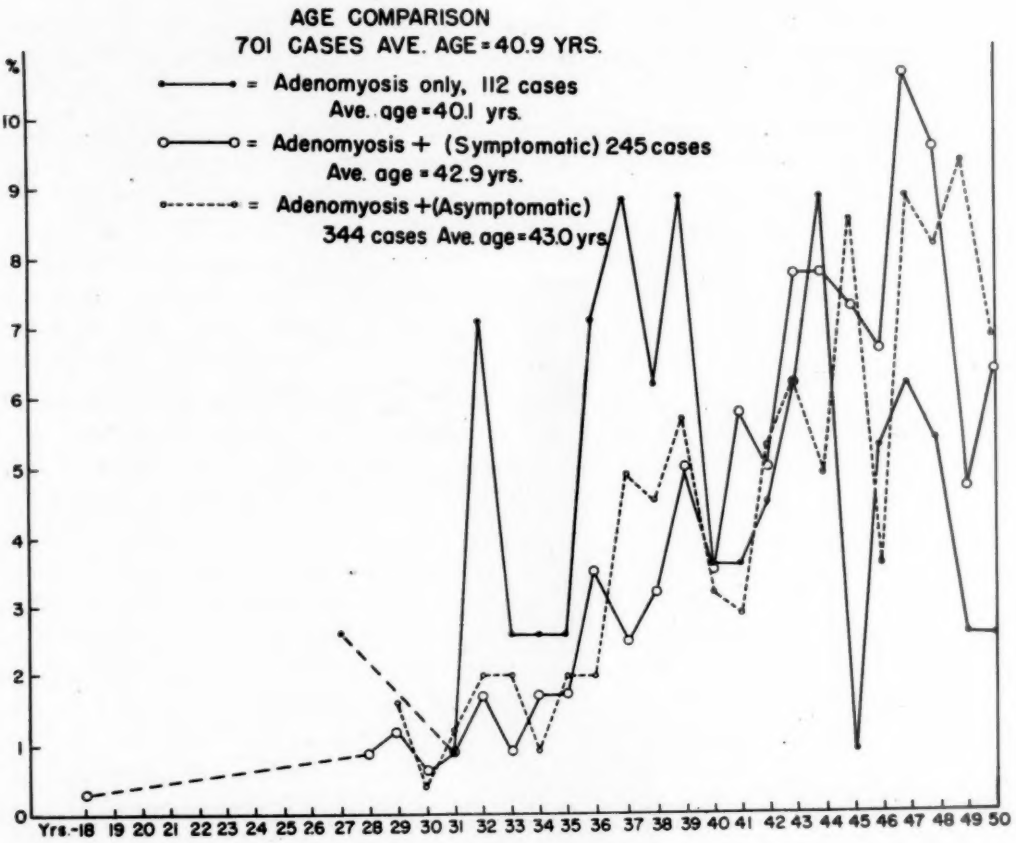

Fig. 2.

TABLE I. PARITY OF PATIENTS WITH ADENOMYOSIS

CLINICAL CATEGORIES	PRIMIPARAS		MULTIPARAS	
	NO.	%	NO.	%
Adenomyosis only (Bleeding, pain)	21	18.0	91	82.0
Adenomyosis + (Bleeding, pain)	84	24.5	260	75.5
Adenomyosis + (No bleeding, pain)	41	16.8	204	83.2
Total patients	146	20.8	555	79.2

Parity.—A comparison of the obstetrical background of the three groups of interest is shown in Table I. Multiparity was three to four times as common as primiparity in the three groups under study.

Menstrual Complaints.—Figs. 3 and 4 depict single and multiple menstrual difficulties in two of the three patient groups. Menorrhagia (hypermenorrhea) and dysmenorrhea were most frequently described by women with adenomyosis only, and adenomyosis plus other conditions also symptomatic of pain and bleeding. This was apparent in those who had either one or more than one complaint.

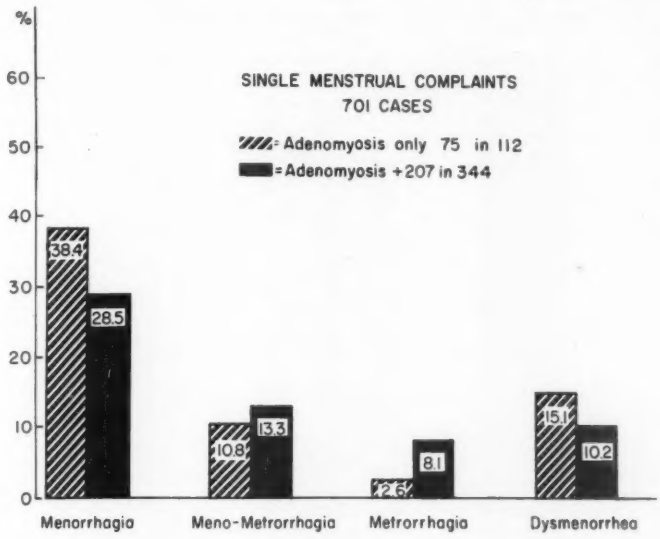

Fig. 3.

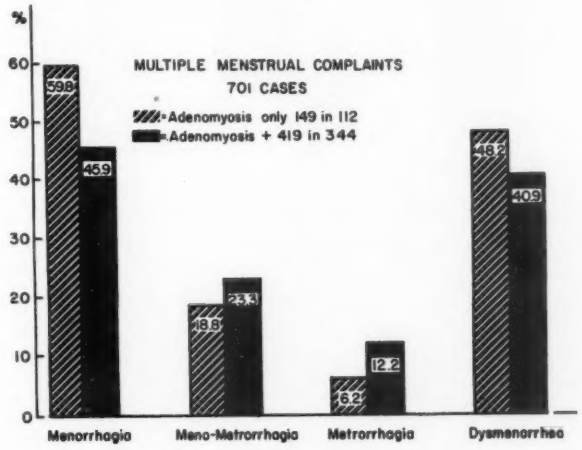

Fig. 4.

Menometrorrhagia was much less frequent in adenomyosis, and metrorrhagic was very uncommon.

Women who reported menorrhagia *and* dysmenorrhea were next culled from each of the two groups: (1) those who had adenomyosis alone (Fig. 5) and (2) those who were found to have adenomyosis together with other significant abnormality capable of causing menstrual changes and dysmenorrhea

(Fig. 6). These cases were then arranged according to age and were also considered with regard to the extent of involvement of adenomyosis. It was found that the number of patients under 30 and over 49 years of age was too small for analysis. The groups from 30 to 39 and from 40 to 49 years were large enough, however, for certain comparisons. It is apparent that, as age increased, the degree of adenomyosis tended to progress.

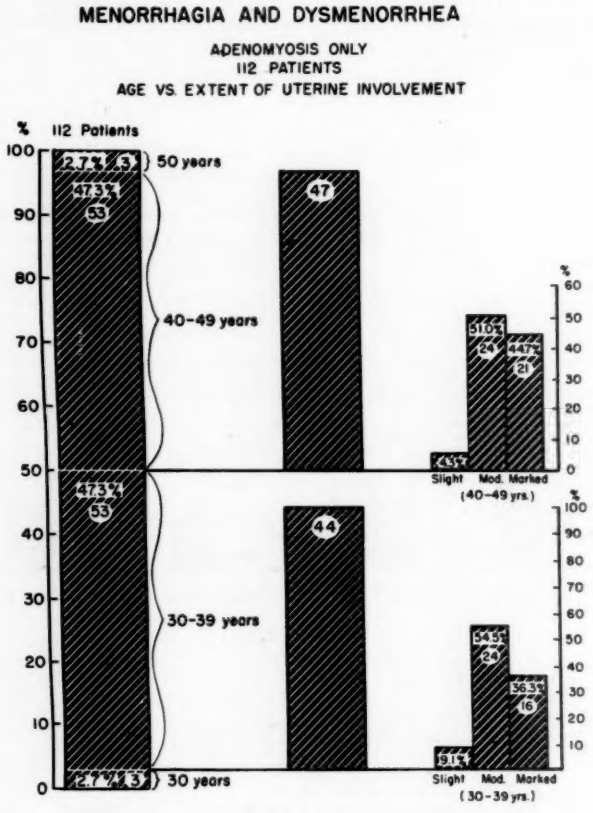

Fig. 5.

Enlargement of the Uterus.—In cases where there was no increase in size of the uterus, asymptomatic adenomyosis was the type most frequently found. This was followed by symptomatic adenomyosis, both singly and with other changes, as shown in Fig. 7. Symptomatic adenomyosis alone was most often found in the slightly enlarged uterus. Adenomyosis in association with other conditions, both symptomatic and asymptomatic, was noted with almost equal frequency with minimal enlargement of the uterus.

In cases where there was a moderate increase in the size of the uterus, adenomyosis alone and with other changes was associated with pelvic pain and/or abnormal bleeding.

Marked enlargement was most often due to the presence of myomas, although adenomyosis was also frequently discovered. Adenomyosis alone was rarely considered to be the sole cause of extreme uterine tumefaction.

Degree of Adenomyosis.—The larger the uterus, the more often adenomyosis was found to be symptomatic, as shown in Fig. 8. We observed that this includes both uncomplicated and complicated adenomyosis with symptoms of unusual bleeding and pain, but the association is more strikingly revealed in the uncomplicated group.

Parity Versus the Extent of Adenomyosis.—Inasmuch as parity might be a factor in adenomyosis, we considered it worth while to appraise multiparity in the three groups of patients under study as it applied to the extent or penetration of the adenomyosis. Fig. 9 indicates that the incidence of childbirth has some bearing on the occurrence of adenomyosis, although grand multiparity, for example, was not associated with extreme involvement. (The small number of patients with more than three children obviously reduces the significance here.)

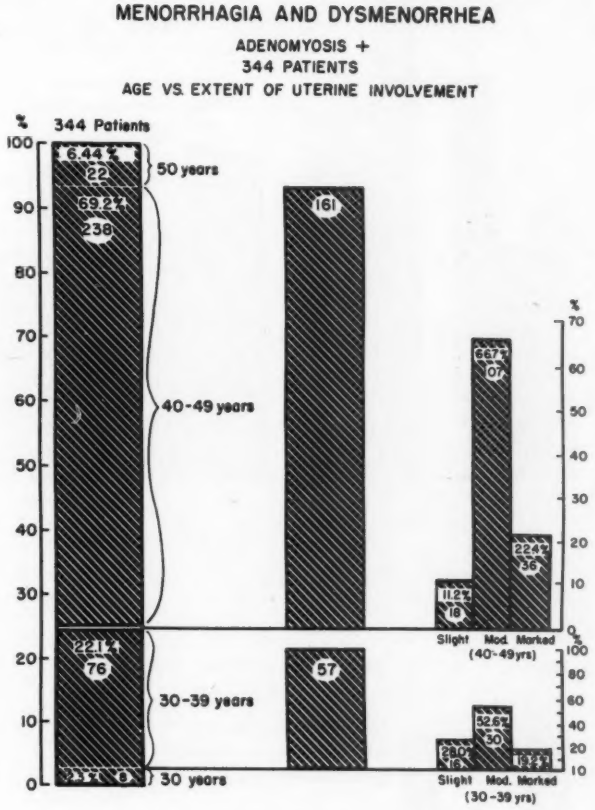

Fig. 6.

Duration of Symptoms Versus Extent of Adenomyosis.—When the reported symptomatology was plotted against the stage of adenomyosis (Fig. 10), moderate and marked invagination of the uterine wall was found to be related to the length of time during which the patient had pain and/or abnormal bleeding. (We found that many patients could not accurately recall the onset of their symptoms, particularly when they had been present for more than 3 years. Hence, only a trend is noted and a further projection is not valid.)

Endometrial Findings (Uterine Cavity) in Patients With Adenomyosis.—A high proportion of patients in the several categories had secretory endometrium (Table II). Women with bleeding and/or pain considered to be due to adenomyosis alone were separated from those who also had polyps, carcinoma, and the like. Yet, even the group of patients with adenomyosis in addition to other symptoms did not disclose persistent proliferative or hyperplastic endometrium, nor did those with asymptomatic pelvic endometriosis.

Myomas, Pelvic Endometriosis, and Salpingitis Isthmica Nodosa.—Leiomyomas were found associated with adenomyosis in 398 cases, or 56.6 per cent of

the total. We noted endometriosis within the pelvis as an associated abnormality with adenomyosis in 93 patients, or 13.3 per cent of the total. The great majority of the cases was in the group with symptomatic adenomyosis with additional significant abnormality.

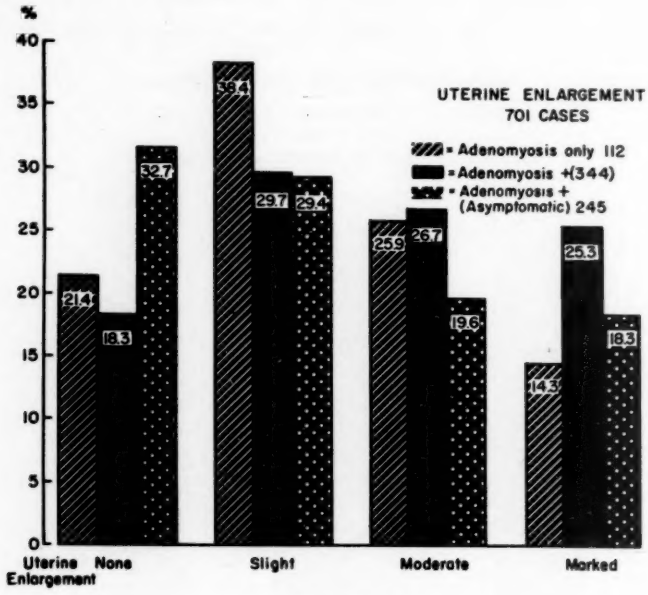

Fig. 7.

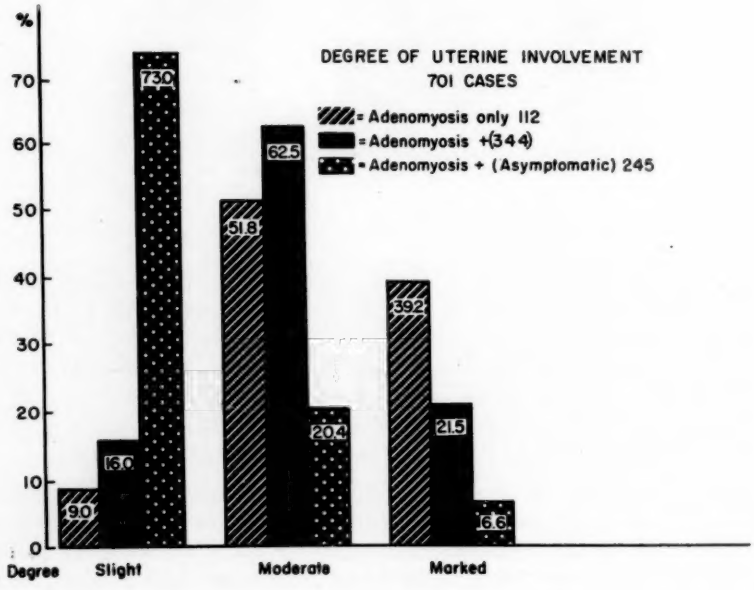

Fig. 8.

Salpingitis isthmica nodosa was discovered in 19 cases, or 19.8 per cent of 96 patients whose operation included the removal of both Fallopian tubes with

TABLE II. STATE OF ENDOMETRIUM IN PATIENTS WITH ADENOMYOSIS

CLINICAL CATEGORIES	NO. OF PATIENTS	ENDOMETRIUM							
		SECRETORY	PROLIFERATIVE	HYPERPLASIA	POLYPS	ENDOMETRITIS	CARCINOMA	OTHER	UNREPORTED
Adenomyosis only (Bleeding, pain)	112	55	57	0	0	0	0	0	0
Adenomyosis + (Bleeding, pain)	344	145	131	51	36	0	8	3	6
Adenomyosis + (No bleeding, pain)	245	109	96	26	19	1	2	5	12
Total patients	701	309	284	77	55	1	10	8	18

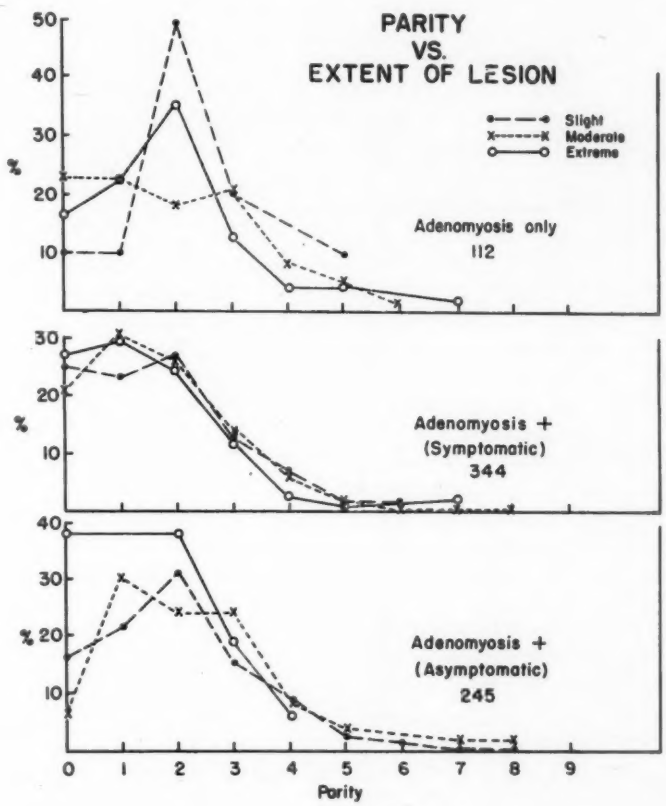

Fig. 9.

the uterus. This condition is almost never seen as an isolated symptomatic entity. In several of our cases, unilateral pain referred to the inguinal region may have had part of its origin in salpingitis isthmica nodosa, however.

Accuracy of Diagnosis of Adenomyosis.—Adenomyosis was mentioned only 65 times in this series by responsible physicians in their preoperative diagnoses in the 701 cases reviewed (Table III). Usually, of course, several possibilities

were recorded. Admitting even the mention of the lesser-choice diagnosis of adenomyosis by generalists and specialists combined resulted in only 9.3 per cent accuracy. Adenomyosis was diagnosed most often (16.9 per cent) when symptomatic and unassociated with other serious problems also causative of pain and bleeding. Asymptomatic adenomyosis was diagnosed correctly before operation in only 5.7 per cent of the cases.

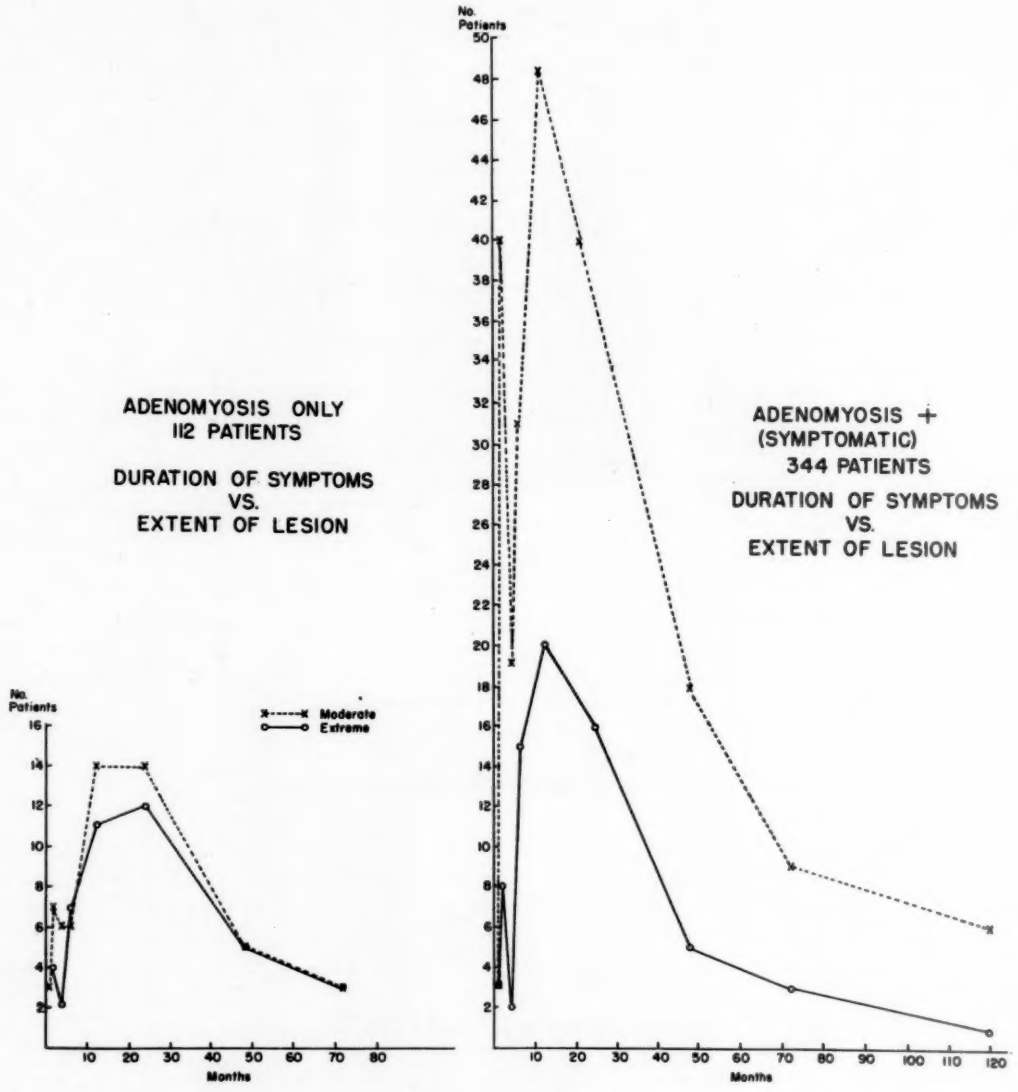

Fig. 10.

Pathologists grossly diagnosed adenomyosis correctly in 66.7 per cent of all these surgical specimens, and in 82.1 per cent of the cases of adenomyosis alone, as shown in Table III. (Regrettably, the accuracy of the surgeons in recognizing adenomyosis at the operating table cannot be appraised from the histories alone. We do speculate, however, that it does not approach that of the pathologist.)

TABLE III. ACCURACY OF DIAGNOSIS OF ADENOMYOSIS

CLINICAL CATEGORIES	NO. OF PATIENTS	BY SURGEON (PREOPERATIVE)		BY SURGICAL PATHOLOGIST	
		NO.	%	NO.	%
Adenomyosis only (Bleeding, pain)	112	19	16.9	91	82.1
Adenomyosis + (Bleeding, pain)	344	32	9.3	264	76.7
Adenomyosis + (No bleeding, pain)	245	14	5.7	113	46.1
Total patients	701	65	9.3	468	66.7

Comment

Adenomyosis as a Pathological Entity.—Adenomyosis can occur in any portion of the uterus as a more or less circumscribed lesion (adenomyoma) or, more commonly, as a diffuse process in several portions of the organ. We have considered these two types together. It is most frequently discovered in the posterior wall, less frequently in the anterior uterine wall, and rarely involving only the cornua or a portion of the uterus near the internal os.

Adenomyosis is a proliferative process, now and then obscured when fibromyomas are present, but usually recognizable on scrutiny. Classically, in the unfixed surgical specimen, the uterus is enlarged, often symmetrically, and is irregularly firm. In addition, increased vascularity of the corpus may be seen. Incision into an involved area may disclose a marked thickening of the uterine wall. Findings are variable but include coarsely trabeculated areas, stippled or granular in appearance, with small yellowish or darker cystic points which may contain serous fluid or old blood. The cut surface appears convex and bulging. An irregularity of the endometrial-myometrial juncture is often apparent, with dipping-down of the lining epithelium of the cavum more or less perpendicularly into the whorled, firm, prominent muscle. The inner aspect of the uterus is always the more seriously involved. It is a diffuse tumor process with ill-defined borders.

Microscopic study confirms the endometrial extension into the myometrium. Invariably, there is an alteration of the latter of a "hypertrophy-hyperplasia" type. In fact, the presence of this feature is required to substantiate the diagnosis of adenomyosis. The ectopic glands generally resemble the basalis, appearing only occasionally responsive to progesterone. The stroma may exceed the glandular elements, especially when the "tumor" is yellow grossly. When there are plugs of this type of material within tissue spaces and perhaps even in vessels, the possibility of this being an area of stromal-cell sarcoma should be considered.[10, 11] Only isolated instances of carcinoma developing in an area of adenomyosis are recorded,[12] so the threat of this type of cancer is not great.

Phagocytized hemosiderin deep in the myometrium may be seen, indicating previous extravasation of blood. Unfortunately, intramyometrial bleeding is only rarely observed during menstruation in adenomyosis, with the commonly used techniques of fixation and section. The use of Bouin's solution fixative insures much better detail and hemosiderin is more often seen. The deeper glands communicate with the uterine cavity and drainage is usually possible.[3] Yet, the absence of gross blood in most of the ectopic gland spaces may well be due to the fact that free bleeding in these areas does not commonly occur.

Salpingitis isthmica nodosa may result from a process of extension of adenomyosis from the cornu of the uterus into the tube in many cases.[13] This

can lead to tubal occlusion on the affected side. The relative rarity of this lesion would rule it out as a frequent cause of infertility in our opinion.

The incidence of adenomyosis (21.4 per cent) seems high in comparison with certain previously published figures.[14, 15, 16] We believe this is because most authors have generally sought adenomyosis in *all* uteri during a particular time period, irrespective of the age and menstrual function of the patients. This increases the age differential, of course, and includes many postmenopausal women, operated upon for complaints other than pain and bleeding. Because of our primary interest in menstrual difficulties and active adenomyosis, we excluded amenorrheic and postmenopausal patients. Had we included every instance of adenomyosis during the same 7-year period from all hysterectomy specimens, the incidence would have been 17.4 per cent. This actually is low in comparison with figures given in other reports.[8]

Clear-cut metrorrhagia was certainly rare in our experience, unless we are required to consider menometrorrhagia in the same category. Excluding this latter subdivision, our findings are the reverse of Jeffcoate's[17] observation that metrorrhagia, rather than menorrhagia, is the most frequently observed sign of adenomyosis.

Cullen[3] formulated the axiom that adenomyosis is the likely diagnosis when dysfunctional bleeding and increasingly severe dysmenorrhea accompany an enlarging, firm, tender uterus. Admittedly, the diagnosis must be a presumptive one until the anatomical studies are completed. Ward and White[18] have stated that adenomyosis should be strongly considered when painful dysfunctional bleeding is reported, especially in the woman who is 40 to 50 years of age. We agree with these authors that this conclusion is rarely achieved in clinical practice. Emge[6] diagnosed and succeeded in confirming the preoperative diagnosis in 65 per cent of his cases, but even the specialists may be overly impressed by irregularity of the uterus or questionable adnexal pathology. As it is, the diagnosis has been "largely guesswork, subject to errors of omission or erroneous consideration."[18] The fact is, adenomyosis is simply not considered preoperatively, as our study of the recorded multiple diagnostic possibilities indicates.

Ovarian dysfunction has been suspected as an etiological factor in abnormal bleeding in adenomyosis,[17, 19, 20] although there is at least one authoritative opinion to the contrary.[21] Notably, suitable controls have been omitted, age limits neglected, and the criterion of ovulation disregarded in most of these studies. Our material does *not* support the contention that there is a high incidence of ovarian failure or "overactivity" in adenomyosis. The inclusion of menstruating patients only not over 50 years of age is probably reflected in our figures. Hyperplasia of the endometrium was noted in only 10.9 per cent of the total (Table II). Moreover, but 14.7 per cent of the patients with adenomyosis plus other conditions capable of causing bleeding and pain, and 10.6 per cent of the women with asymptomatic adenomyosis were shown to have hyperplasia of the endometrium. The finding of secretory endometrium in 44.0 per cent, 49.1 per cent, and 44.4 per cent, respectively, in our three categories attests to continued ovulation in many, despite the fact that almost four-fifths of the total were 40 to 50 years of age.

Ten cases of endometrial carcinoma were noted, but none of these appeared to have arisen in an area of adenomyosis. We have included no instance of stromal-cell sarcoma in this series, although there were 9 such cases during this same 7-year period.

Adenomyosis as a Cause of Bleeding and Pain.—The basis for the hypermenorrhea in adenomyosis is still debated. Intramyometrial bleeding is uncommonly seen in adenomyosis, but it does occur. We believe that the mere

increase in endometrial surface may not be all important in itself, because the greater part is buried and not morphologically functional. Actually, if bleeding were abnormally brisk from only the superficial areas of the cavity itself, hypermenorrhea would result.

We find that the vascular supply to the uterus may be appreciably increased in many cases of adenomyosis and enormously so in markedly involved cases. Robert Meyer[22] held that faulty vascular control due to interference with myometrial contractility is the basis of the excessive bleeding in adenomyosis. Distention of the scarred, honey-combed uterine wall by this increased vascularity during the menses would also explain dysmenorrhea in this condition. The fact that the process takes time to become clinically apparent, and yet is progressive only to the menopause, further explains the symptomatology.

This would be a neat package indeed if it were not for the fact that about one third of the cases of adenomyosis are either atypical or asymptomatic and discovered by accident. No one has explained why dysmenorrhea, like abnormal bleeding, occurs in some but not in other women with similar abnormalities. Despite these deficiencies, we *can* diagnose the majority of cases correctly if we try.

One must continue to speculate upon the cause of adenomyosis. This we all have done with regard to myomas. In adenomyosis, one wonders why the endometrium seeks to extend into the myometrium, because its benign appearance is so obvious. Could it be that, after advanced pregnancy, the postpartum uterus contracts with an infolding of islands of endometrium? But this does not account for adenomyosis in patients in whom pregnancy has never occurred.

However this may be, myomas and adenomyomas usually present a very *different* symptomatology. Of course, both can develop in the same patient and cause somewhat similar complaints. Yet they can and should be differentiated one from the other. The history of progressive menstrual excess, pain, and firm, nontender enlargement of the uterus might suggest a submucous myoma. Metrorrhagia, however, is more common than menorrhagia with a fibroid. Moreover, unless the myoma is pedunculated or in the process of infarction or extrusion, pain is not a serious problem. Dilatation and curettage will usually reveal even a partially submucous tumor, and most carcinomas. Yet, in this series, many patients with adenomyosis had had repeated curettage for abnormal bleeding, pain, and a presumed myoma, with the findings of only slight enlargement and a somewhat voluminous uterine cavity. Of significance too, no relief of symptoms occurred in these cases after curettement.

Idiopathic hypertrophy of the uterus ("fibrosis uteri") must be considered in the differential diagnosis, but this entity is not uniquely symptomatic.

Another problem might be the pelvic congestion syndrome as described by Taylor[23] which is characterized by a history of chronic, almost continuous pelvic discomfort and menometrorrhagia, and a slightly enlarged, symmetrical, and questionably softened uterus. The cervix is cyanotic and softened also. Hence, a differentiation of this condition from adenomyosis is possible, but frequently difficult.

We find that hysterosalpingography may disclose myomas, but adenomyosis is rarely shown. In our experience, estrogen and androgen therapy has very little effect on the amount of bleeding or pain in adenomyosis (unless amenorrhea is produced). The symptomatology of myomas can occasionally be more favorably influenced.[24] Nevertheless, the blood vessel dilating effect of estrogen may cause appreciable softening and tenderness of an area of adenomyosis (Halban's[25] sign). Myomas are unaffected, however.

Myomas and adenomyosis will frequently be found in association, we admit. The presence of numerous small, or even larger, discrete fibroid tumors should not obviate significant adenomyosis, if the history and other findings are indicative of the latter.

Endometriosis or salpingitis can also occur with adenomyosis. Where pelvic induration, nodulation, and adnexal tenderness are met, these possibilities must be considered. Nevertheless, most cases of adenomyosis should not be obscured by these entities. Usually, the symptom complex of menstrual pain, abnormal bleeding, and an enlarged, firm, tender uterus will be significant in its own light—and adenomyosis will be the correct diagnosis.

Summary and Conclusions

1. Seven hundred and one consecutive instances of adenomyosis in menstruating women 18 through 50 years of age were treated at the Emanuel Hospital, Portland, Oregon, during the years 1950-1957, inclusive. The incidence of adenomyosis in hysterectomy specimens during this time was 21.4 per cent in women of this age distribution.

2. An appraisal of the signs and symptoms of adenomyosis was made with reference to age, parity, menstrual difficulties, enlargement of the uterus, extent of adenomyosis, and associated medical, surgical, and gynecological disorders. The likelihood of adenomyosis as a cause of the symptomatology was estimated.

3. Three groups of patients were compared: (a) those found to have symptomatic adenomyosis only; (b) those with abnormal uterine bleeding and pelvic pain with adenomyosis—together with other conditions also capable of causing these difficulties; and (c) those with asymptomatic adenomyosis whose uterine disease was discovered incidentally at operation.

4. Menorrhagia was the most common sign, and dysmenorrhea was the most usual symptom of adenomyosis.

5. Symptomatic adenomyosis was most often found in the slightly or moderately enlarged uterus. As the size increased, other associated pathological conditions (myomas, etc.) became notable. Adenomyosis was rarely found to be the sole cause of marked enlargement of the uterus.

6. As age increased, the stage of adenomyosis tended to progress. Parity was also related to the extent of adenomyosis, to a degree, as was the duration of the symptoms of pain and/or bleeding.

7. Myomas were very frequently associated with adenomyosis, but hyperplasia of the endometrium and pelvic endometriosis were not commonly noted.

8. Despite the fact that the symptomatology should have led to a preoperative diagnosis of adenomyosis in many instances, it was diagnosed in less than 10 per cent of the cases before operation. On the other hand, the surgical pathologist made the diagnosis in 66.7 per cent of the gross specimens.

9. Adenomyosis is a serious, progressively disabling disorder of the premenopausal woman. Our experience has confirmed its traditional symptomatology.

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Discussion

DR. GEORGE H. GARDNER, Chicago, Ill.—This is a study of the symptomatology presented by 701 women who were no older than 50, who were still menstruating, who had had hysterectomy at the Emanuel Hospital in Portland during the past 8 years, and whose uteri contained adenomyosis. Hence, this was a selected series of patients and on that basis the incidence of uteri with adenomyosis was 21.4 per cent, whereas among all uteri removed during those 8 years the incidence was 17.4 per cent. It is not clear to me why this study was limited to women of this particular age group, since all with adenomyosis not only have not yet stopped menstruating by age 51 but also all do not necessarily have symptoms.

In his original monograph, Cullen reported an incidence of only 5.7 per cent and one third of his patients were over 50 years of age. Nevertheless, I must emphasize that Dr. Benson's criteria for the diagnosis of adenomyosis are unusually rigid.

According to Cullen, the clinical picture varied with the location of the growth and with the size and situation of the myomas which so often accompany it. It was also Cullen's opinion, however, that the diffuse type of growth per se led to lengthened and profuse menses, which were accompanied by a great deal of pain, and gave rise to moderate enlargement of the uterus which was sometimes hard. With all of that Benson apparently agrees, but he has gone further and has attempted to relate the specific pathological conditions more accurately to the patient's complaints, after eliminating factors which of themselves can cause both bleeding and pain.

On the basis of their symptoms and any associated disease, his 701 cases are divided into the following three groups: (1) 112 cases, or 16 per cent, in which adenomyosis alone was thought to be responsible for symptoms; (2) 245, or 35 per cent, in which the adenomyosis apparently had not caused symptoms, and (3) 344, or 49 per cent, in which the adenomyosis was considered to be no more than a contributory factor in the patient's complaints.

To me, the foregoing division of cases is devastating to one of the objectives of this paper, namely, earlier and more accurate clinical diagnosis of adenomyosis uteri. In half of the cases where it was symptomatic, there were other pathological conditions as well, and in the remainder only one in 3, or 16 per cent of the total group, presented classical symptoms.

Furthermore, in those with classical symptoms and no associated pathology there was significant enlargement of the uterus in only 45 cases. This is an incidence of only 6.4 per cent of the entire series where symptoms and physical signs should have resulted in an accurate clinical diagnosis.

Benson comments on the infrequency with which adenomyosis uteri (internal endometriosis) and pelvic or external endometriosis are coexistent, and with that I am in accord. He found this to be true in only 13.3 per cent of his cases. With us, Bayly and Yates found both present in only 17 per cent of 103 cases of adenomyosis which were studied in respect to the symptom of bleeding. Incidentally, 49 per cent of that group had not had abnormal bleeding.

The author states: "Pathologists grossly diagnosed adenomyosis correctly in 66.7 per cent of all these surgical specimens, and in 82.1 per cent of the cases of adenomyosis alone. . . . Regrettably the accuracy of the surgeons in recognizing adenomyosis at the operating table cannot be appraised from the histories alone." One can only wonder whether those surgeons had an opportunity to inspect the opened uterus at the operating table. Adenomyosis cannot be recognized in the unopened uterus, although it can be recognized if looked for in the walls of the opened specimen because of the diffuse type of tumefaction and not because of conspicuous blood-filled cysts therein which actually are quite infrequent.

Benson has commented on the almost universally inactive state of the endometrium in adenomyosis. He has also expressed wonderment not only that so many of his cases were asymptomatic but also that adenomyosis should ever give rise to profuse, excessive menstrual bleeding. Further, although he has told us of the phase of development of the endometrium lining the uterus, at no time has he mentioned the actual phase of development of the endometrium in the adenomyosis. This may be highly pertinent in the explanation of the symptomatology of all types of endometriosis, both the external as well as adenomyosis. With us, Dr. William Roach, in a report not yet published of 179 cases of adenomyosis studied with this particular point in mind, found that the normally located endometrium was secretory in 41, but, of these, it was secretory in the aberrant islands in only 19; 12 times it was menstrual inside the uterus but the islands were menstruating in only 6; one of his patients was pregnant and there were decidual changes in the endometrium of the adenomyosis. In other words, Roach found functional response in the aberrant endometrium in only about 50 per cent of the cases where the normally located endometrium was functional. Symptoms probably should not be anticipated if and when the aberrant islands are nonresponsive to progesterone.

DR. S. LEON ISRAEL, Philadelphia, Pa.—The fact that the adenomyotic process is much more common than is generally realized has been amply illustrated by Drs. Benson and Sneeden who cite 245 women in whom asymptomatic adenomyosis was discovered "quite by accident" when hysterectomy had been performed for descensus. In an as yet incomplete retrospective survey of adenomyosis under way in our two hospitals, we have found that nearly two thirds of the instances diagnosed initially in the removed uterus had created no recorded symptom. This high incidence of asymptomatic adenomyosis accounts, in part, for the diagnostic supremacy of the pathologist. The divergency is widened by the gynecologist who fails to record preoperatively his unvoiced suspicion of adenomyosis. The authors hint at this shortcoming of preoperative notes by stating parenthetically, "Regrettably, the accuracy of the surgeons in recognizing adenomyosis at the operating table cannot be appraised from the histories alone." In other words, a reviewer of the records cannot read what might have been in the mind of the gynecologist in charge. One need do only one such record room chore to appreciate the value of notes which describe symptoms and include a preoperative diagnosis. As pointed out by the essayists today in their distillation of the "pure" or uncomplicated entity and by Cullen 50 years ago, the diagnosis of adenomyosis is "relatively easy" because of its two symptoms, menorrhagia and dysmenorrhea.

We cannot but agree with the conclusion of Drs. Benson and Sneeden who, having reappraised the traditional symptomatology of adenomyosis, find it "a serious, progressively disabling disorder of the premenopausal woman." It would be well perhaps to add as an addendum, suggesting that much more remains to be done before we understand the full consequences, the last sentence of Cullen's classic: "The cause of adenomyosis is still unsolved."

DR. LUDWIG A. EMGE, San Francisco, Calif.—Dr. Benson showed that in a mixed group of practitioners the recognition of adenomyosis is below 10 per cent. In contrast, an analysis of my private material, consisting of 212 instances of adenomyosis encountered in 1,412 total hysterectomies, shows that a clinical diagnosis was correctly arrived at in 64.6 per cent as reported by me in 1955 (West. J. Surg. 64: 291, 1956). The explanation rests on the fact that an adequately long observation of changes in configuration and consistency of the uterus, the absence of surgical haste, and a sound understanding of the nature of dysfunctional bleeding and progressive dysmenorrhea will make it possible to arrive at a correct diagnosis of adenomyosis with much greater frequency than was recorded by Dr. Benson. Errors will occur in either direction because other uterine conditions, such as idiopathic hypertrophy with minimal stromal invasion or such obscuring accompaniments as multiple fibroids and endometriosis externa, will defeat the most painstaking attempts at differentiating symptoms. My diagnostic achievement is recorded in Table I.

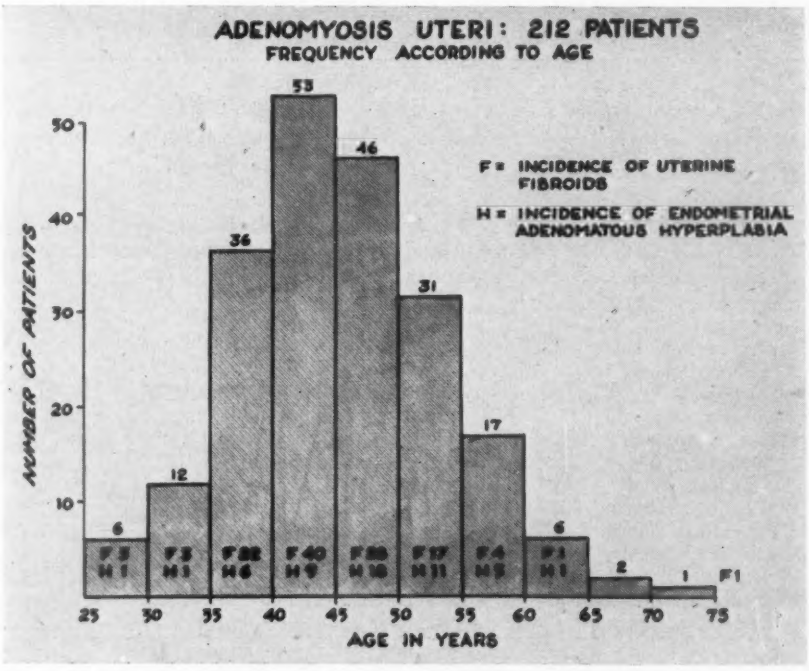

Fig. 1.—From Emge: West. J. Surg. 64: 291, 1956.

TABLE I

	NO.	%
Material surveyed:	1,412 total hysterectomies	
Proved incidence of adenomyosis	212	15
Presumptive diagnosis	147	
Histologically confirmed	95	64.6
Incidental laboratory diagnosis	117	55.2

The greatest number of correct diagnoses were recorded in patients between the ages of 35 and 55. It is noteworthy that endometrial adenomatous hyperplasia and uterine fibroids also had the highest incidence in this age group which suggests a common etiological background.

Dysfunctional bleeding and dysmenorrhea no doubt aid in diagnosing adenomyosis but, according to my observations, not as frequently as was originally stated by Cullen. My own observations are recorded in Table II.

TABLE II. DYSFUNCTIONAL BLEEDING AND STATE OF THE ENDOMETRIUM IN 212 CASES OF ADENOMYOSIS UTERI

	AGE OF PATIENTS				TOTAL	
	UNDER 35	35-44	45-54	55 AND OVER	NO.	%
Dysfunctional bleeding	15	49	43	4	111	52.3
Postmenopausal bleeding	–	–	2	15	17	8.0
Dysmenorrhea	17	30	10	1	58	27.4
Normal endometrium	17	62	45	1	125	58.9
Atrophic endometrium	–	2	6	11	19	8.9
Adenomatous hyperplasia	2	15	29	5	51	24.0
Polyps and normal endometrium	1	5	7	4	17	8.0
Carcinoma	–	2	2	9	13	6.0
Fibroids	10	62	45	6	123	58.0
Adenomyosis, uncomplicated	7	20	15	8	50	23.6
Endometriosis, external	7	18	7	–	32	15.1

Evidently, a considerable number of uteri harboring adenomyosis become asymptomatic or, as stated before, the symptoms are completely overshadowed by those of other pathological conditions (Table III).

TABLE III. PATHOLOGICAL CONDITIONS WHOSE SYMPTOMS OVERSHADOWED THOSE OF ADENOMYOSIS UTERI IN 117 CASES NOT DIAGNOSED CLINICALLY BUT DISCOVERED MICROSCOPICALLY

		NO.	%
Uterine fibroids		52	44.44
Uterine malignancies		13	11.11
Corpus cancer	10		
Sarcoma	1		
Cervix cancer	2		
Ovarian neoplasms		15	12.82
Benign types	13		
Cancer	1		
Lecène's tumor	1		
Pelvic endometriosis		17	14.53
Chronic pelvic inflammatory disease		7	5.98
Tuberculous endometritis		1	0.85
Procidentia uteri		12	10.27
Total		117	100.00

Neither uterography nor deep curettage has been of sufficient diagnostic assistance to me to warrant the expense entailed. Estrogen trials, however, have been of material help and are in line with Halban's original observations.

DR. BENSON (Closing).—The reason for limiting the patients as we did to the age of 50 years was to try to exclude women who had other problems and perhaps to exclude some of those we thought might have been subjected to hormone therapy. We wanted to avoid inclusion of many of the dysfunctional bleeding problems which come into the picture at the menopause. I will agree that our division of cases detracts to some extent from the

percentage differential diagnosis. If, however, we had lumped together the adenomyosis only cases and those with adenomyosis plus other conditions capable of causing pain and uterine bleeding, we would have had a more striking percentage of both signs, symptoms, and results.

I think the suggestion of investigation of the function of the deeper glands may be very worth while indeed. Although I was not impressed at first with what these glands were doing, I think the only way to know will be to lay them all out and see.

I too want to pay tribute to Dr. Thomas Cullen. I personally did not doubt the existence of this symptomatology, but I thought it was worthy of review, particularly because of poor diagnosis in most general hospitals. As Dr. Israel and Dr. Emge stated, the specialist thinks of this but the generalist and general surgeon still do not.

We were impressed with the fact that von Recklinghausen had only four cases of adenomyosis and most of them were associated with marked endometriosis, so his inclusion of this condition among others probably does not warrant nearly the recognition which we give to Dr. Cullen.

I believe the mere presence of myomas or hyperplasia of the endometrium should not deter us from considering adenomyosis as a possibility in dysfunctional bleeding, and I believe that if we remember adenomyosis in our differential diagnosis the accuracy of diagnosis will be improved.

THE USE OF HYPOTHERMIC-HYPOTENSIVE TECHNIQUE IN FULMINANT TOXEMIA OF PREGNANCY*

ROBERT H. BARTER, M.D., SALOMON N. ALBERT, M.D., AND ALAN W. WINSHEL, M.D., WASHINGTON, D. C.

(From the Departments of Obstetrics and Gynecology, and Anesthesiology, District of Columbia General Hospital and The George Washington University)

ALTHOUGH the etiology of acute fulminant toxemia of pregnancy continues to be an unsolved problem, there have been some rather significant changes in its therapy during recent years.[1, 2] The antitensive drugs and the newer diuretics have provided significant contributions to the therapeutic armamentarium of the obstetrician in the treatment of acute toxemia.[3-6] The fact remains, however, that all medical therapy is still of limited value. To date, the only satisfactory treatment of acute toxemia is the termination of the patient's pregnancy. Over the years it has become increasingly evident that procrastination in emptying the uterus leads only to an increase in complications.[7]

One of the greatest problems of operative delivery is that the patient with acute toxemia of pregnancy has always been, and continues to be, an extremely poor surgical and anesthetic risk. That remains a pertinent fact whether the patient is to have an operative vaginal delivery or whether she is to be delivered by cesarean section.

If the concept of prompt termination of pregnancy in acute toxemia is valid, a concerted effort must be made to make the operative delivery safer for the patient and her unborn fetus. The hypothermic-hypotensive technique described in this presentation was introduced experimentally as a means of giving added protection to both the patient and the fetus from the surgical trauma of an operative delivery. Strangely enough, the use of cold in obstetrics is not new. "Cooling the head" in toxemia patients was recommended by Gooch of London as early as 1832.[8] "Refrigeration of the head" in the treatment of convulsions of pregnancy was mentioned in 1834 by James Blundell of London.[9] The use of hypothermia in surgery was suggested by Fay[10] in this country about 20 years ago. Refrigeration in the treatment of obstetric patients in shock from hemorrhage was described by Allen[11] in 1948, but the technique described was much too cumbersome for its general adoption. The patients described herein may represent the first use of hypothermia in the management of patients with acute toxemia of pregnancy. The addition of active hypotensive therapy through the use of a ganglion-blocking agent to facilitate the hypothermia was recently suggested by Eccleston and co-workers[12] and by Albert and his associates.[13]

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The first obstetrical patient to be treated at the D. C. General Hospital with hypothermic-hypotensive technique was a patient who had a puerperal gas bacillus infection. It was the opinion of the staff that the patient survived the infection and the subsequent operation primarily because of the use of hypothermia. Since the patient with acute toxemia is also an extremely poor surgical candidate, it was postulated that the hypothermia might be of great value in the operative treatment of that condition. Accordingly, its use was proposed in the treatment of a few selected patients with acute toxemia. The results obtained in the patients thus treated comprise the basis for this preliminary report.

Method

In the 18 month interval between Sept. 1, 1956, and Feb. 28, 1958, during which this study was carried out there were 9,300 patients delivered at the D. C. General Hospital. Of these, 1,144 had toxemia of pregnancy. There were 11 patients who had eclampsia.

The hypothermic-hypotensive technique was used in 10 cases of acute toxemia. The ages of the patients under consideration ranged between 13 and 24 years. Nine were Negroes and one was white. Seven were primigravidas, one a secundigravida, and 2 were multigravidas. Five of the patients had unequivocal eclampsia and 5 had pre-eclampsia of a severe degree. Three of the latter also had serious underlying hypertensive vascular disease. There was one multiple pregnancy. The duration of pregnancy varied between 26 and 40 weeks. Eight of the patients were delivered by cesarean section and 2 were delivered vaginally. The indication for cesarean section in 7 cases was acute toxemia in the presence of an unfavorable cervix, and in one case a combination of acute toxemia and cephalopelvic disproportion. The average duration of the hypothermic process (including cooling and warming phases) was 9 hours. The hypothermic level ranged between 30.5° C. and 33° C. at the time that the operative procedures were done.

These patients had the usual diagnostic laboratory studies on admission to the delivery suite, which included a blood urea nitrogen, carbon dioxide combining power, blood chlorides, and blood sodium and potassium determinations. The urine was cultured and was examined for proteinuria. After initial sedation with 250 mg. of pentobarbital sodium (Nembutal) given intravenously, the patients were evaluated from an obstetric standpoint by an aseptic vaginal examination. Electrocardiographic studies were carried out. Five of the patients had blood volume and cardiac output determinations prior to and during the course of the hypothermia.

When it was decided that the hypothermic-hypotensive technique was to be used, the patient was moved to an isolated room equipped with oxygen, a suction machine, a tracheotomy set,[14] an electrocardiograph, and all necessary emergency medications. She was next placed on a Davol mattress which extended from the base of the neck to the upper quarter of the thighs. Electrocardiographic leads were attached for continuous monitoring. Two venepunctures with 18 gauge needles were carried out and slow drips of 5 per cent dextrose in water were started. A 1:500 infusion of trimethaphan (Arfonad*) was prepared and another infusion of Neo-Synephrine containing 20 mg. in 500 c.c. of normal saline was made ready. These two solutions were connected to the infusion system with appropriate stopcocks so that they could be administered when necessary. A

*The Arfonad used in this study was supplied by Hoffmann-La Roche, Inc.

thermocouple was inserted in the rectum for continuous temperature recording. A Foley catheter which had been placed in the urinary bladder previously was connected to a flask so that the urinary output could be measured accurately.

The cooling procedure was initiated by the slow intravenous injection of 25 mg. of chlorpromazine (Thorazine). After 5 minutes, fractionated doses of a solution of 2.5 per cent Pentothal Sodium were administered. Cooling by ice water pumped through the mattress was started as soon as the patient was anesthetized. Ice packs were also placed over the anterior surfaces of the body. Arfonad in 1:500 infusion was administered slowly to maintain a steady fall in the blood pressure. Because of the vasodilatation brought about by the Arfonad, the cooling process could be accelerated. The cardiac rate and rhythm were under constant surveillance. The systolic blood pressure was maintained above 90 mm. of mercury. In the event of a sudden drop in blood pressure the Arfonad was stopped and the Neo-Synphrine solution was substituted. Shivering was controlled by additional doses of a 2.5 per cent solution of Pentothal Sodium or by supplemental doses of 10 mg. of Thorazine given intravenously. The rate of cooling was 2 to 3° C. per hour.

The fetal heart rate was checked frequently. If the patient was in labor, aseptic vaginal examinations were carried out at appropriate intervals.

The cooling process was interrupted as soon as the rectal temperature reached 34° C. The patient was then transferred to the operating room for vaginal delivery or for cesarean section.

A 2 to 3° C. downward "drift" in temperature occurred in all patients. On the average it took 2 hours to obtain the necessary level of refrigeration. For the operative procedures anesthesia was induced and maintained with a 3:2 ethylene-oxygen mixture. Rewarming was allowed to proceed spontaneously after the operative procedure had been completed. The average time for return to normothermia was 4 hours.

In the patients who were in labor there appeared to be no deleterious effect on the labor by the hypothermia.

Following delivery, and as the patients were being warmed, the Arfonad infusion was continued. Prior to the return to normothermic temperature active antitensive therapy was begun. That usually included the administration of reserpine (Serpasil), 2.5 mg. intravenously or intramuscularly, and cryptenamine (Unitensen), 0.5 to 1 mg. intramuscularly. The Unitensen was repeated each hour as necessary to control the blood pressure level. In the case of shivering during the recovery the patients were given Thorazine, 25 mg., or Nembutal, 100 mg., intravenously.

Results

All 10 of the patients in this series tolerated the hypothermia and the hypotension without untoward effects, and all made uneventful recoveries. There were no arrhythmias and no patient developed ventricular fibrillation.

All of the infants weighing over 1,500 grams survived. The only death was that of a fetus whose birth weight was less than 500 grams. The pregnancy in that instance was interrupted at 26 weeks because of severe hypertensive cardiovascular-renal disease with superimposed pre-eclampsia. Only one patient had a convulsion following the return to normothermic temperature. The convulsion in that instance was readily controlled by sedation with intravenous Nembutal and by an increased dose of the antitensive drugs.

Much less anesthesia was used in addition to the hypothermia than would normally have been required for an operative delivery.

No consistent alterations in blood chemistry were noted. Before hypothermia 2 patients had low potassium levels, 3.0 mEq. and 2.6 mEq., respectively.

The latter patient continued to show low serum potassium levels during and after anesthesia. Three other patients had potassium levels of 3 to 3.5 mEq. during or after hypothermia. One significant alteration in acid-base balance was noted. That was in a patient who had a posthypothermia carbon dioxide combining power level of 21 vol. per cent, which responded readily to the administration of ⅙ molar lactate.

The average urinary output before hypothermia for 5 patients was 44 c.c. per hour. The average urinary output during hypothermia for all patients was 73 c.c. per hour, and following hypothermia was 77 c.c. per hour.

All of the infants had the same temperatures as their mothers at the time of delivery. The fetal heart rate was noted to be within normal limits, but the fetal respirations were quite slow, varying between 3 and 4 per minute. The color of the infants remained good, however, in spite of the diminished respiratory rate. All of the infants breathed spontaneously.

Comment

The use of hypothermia as a therapeutic measure in the poor risk obstetric patient seems logical for it has been shown previously that hypothermia may be of extreme value in the management of the poor-risk patient undergoing major surgery.[15-18] Its particular application in obstetric practice appears to be to the patient who is in poor condition from a surgical physiologic standpoint, but in whom obstetric surgery is deemed mandatory.

Three conditions in which this might be applied to obstetrics are: (1) severe toxemia of pregnancy which requires surgical delivery of the patient; (2) an overwhelming intrapartum or postpartum sepsis in which pelvic surgery is indicated; and (3) shock from acute blood loss. The last indication, although advocated by Allen,[11] appears to be of doubtful value. The use of hypothermia as described in this paper is restricted to the first type of patients, namely, those with severe toxemia of pregnancy in whom operative delivery is indicated.

In former years the time required for medical treatment to become effective in the patient with acute toxemia was much longer than that now required with the newer antitensive preparations.[19] As mentioned previously, however, the so-called "conservative" medical management of the patient with acute toxemia of pregnancy is at best a temporizing measure. There is no drug available at the present time which will "stabilize" the acutely toxic patient for more than a relatively short period of time. Therefore, in the absence of labor and in the presence of an unfavorable cervix, cesarean section as a means of terminating the pregnancy assumes tremendous importance in the treatment of this disease.[20, 21] Procrastination in attaining the ultimate goal, namely, the evacuation of the patient's uterus, may lead only to further complications. "Stabilization" of the acutely toxic patient for a "required" length of time, such as 24 to 72 hours, before the pregnancy can be terminated is no longer tenable at the D. C. General Hospital. The concept which has evolved there over the past 10 years is that cesarean section in selected patients is perhaps the most important part of the therapy of this usually preventable, always, hazardous, and too often fatal disease. It has been shown that the fetal survival rate is improved when the infant is delivered without delay from the uterus of an acutely toxic patient.[22]

With this philosophy in mind, we have applied the hypothermic-hypotensive technique with the splendid cooperation of the anesthesia department in an endeavor to make the operative delivery as safe as possible. The use of hypothermia in pregnant patients has been a significant departure from the past. As yet, its future applicability remains unknown in this field.

Previous work with hypothermia has disclosed the following factors of significance:

1. Hypothermia reduces cellular metabolism and thereby protects the vital organs from an otherwise serious reduction in oxygen delivery.[13, 23]

2. More specifically the significant reduction in cerebral oxygen consumption protects the central nervous system from any reduction in blood pressure which would otherwise lead to irreversible brain damage.[24]

3. There is generalized peripheral vasoconstriction under hypothermia with increased peripheral vascular resistance.

4. An occasional complication of induced hypothermia is that of cardiac arrhythmia or fibrillation.[12, 13, 17]

5. With the decrease in metabolism, reduced amounts of anesthetic agents (approximately one half) are required.[13]

6. With the reduction in temperature there is a loss of salt and water, but no change in potassium. Following operation instead of the usual retention there is salt loss.

7. The urinary output is generally increased during and after hypothermia.

8. The stress reaction appears to be delayed.[12, 13]

9. Hypothermia significantly reduces the size of the brain. In this way the extracerebral space in the cranium is increased by as much as 31.8 per cent at 25° C.[24] This may be a factor of great significance in the patient with acute toxemia of pregnancy who may have a considerable degree of cerebral edema.

10. It has been shown that young mammals safely tolerate chilling to much lower temperatures than do the adults of the same species.[25] That fact seems to apply to the newborns involved in this study.

The addition of the hypotensive technique to the cooling procedure was developed for several reasons. They are:

1. To protect the patient against cardiac arrhythmias. It has been shown that hypothermia is associated with an increased incidence of ventricular fibrillation because cold depresses the parasympathetic system earlier than it does the sympathetic system.[17] With the use of ganglion-blocking agents it has been found that the incidence of cardiac arrhythmias is greatly decreased and that the myocardium is protected against functional disorders and a reduction in oxygen supply.[12, 13]

2. With the use of the hypotensive technique the generalized peripheral vasoconstriction which generally results from hypothermia can be overcome.[18] That is particularly important in a patient with toxemia of pregnancy whose disease is due in part to generalized vasoconstriction.[20, 26] The production of active vasodilatation blocks the reflex vasoconstriction caused by the cold and favors an increased peripheral blood flow. The vasodilatation facilitates the cooling process and at the same time reduces the oxygen consumption in the tissues. The latter is particularly important in the central nervous system for the amount of anesthesia necessary to produce unconsciousness and analgesia is thereby greatly diminished.[13]

3. The hypotensive technique also tends to eliminate the shivering mechanism which is in itself calorifical. Shivering also increases oxygen consumption and tends to exhaust the patient rather rapidly.[18]

4. The addition of the hypotensive technique further reduces blood loss, thus making the operative procedure less traumatic.

5. The hypotensive-hypothermic technique greatly reduces the stress reaction.[13] Since the patient with acute toxemia is always under severe stress the recuperative powers of the body can be preserved by the induction of a state of hypometabolism, such as occurs in hypothermia.

Perhaps the most significant finding in this study and one of the least expected was the remarkable fetal salvage. When this project was planned, the possible effects on the fetus were unknown. Thus, it was with some trepidation that the study was undertaken, principally because of the possibility of adverse effects upon the fetus. Although this is an extremely small series of patients, the 100 per cent survival of the 10 infants weighing over 1,000 grams is worthy of note. Previous studies have shown the fetal mortality to be as high as 33 per cent when cesarean section is employed in the management of the patient with acute toxemia.[19, 27]

The hypothermic baby should be allowed to regain its normal temperature without the application of external heat, and overzealous nursery personnel should be cautioned against warming the baby artificially. This is true even for premature infants. The baby with hypothermia tolerates any drug which it has acquired through placental transfer much better by virtue of its hypometabolic state. The reduction of the fetal respiratory rate to 3 to 4 per minute at the time of birth has been a most interesting observation. In spite of the low respiratory rate the babies had excellent color, were not hypoxic, and were in no distress.

There are hazards in the application of hypothermia. The greatest danger is the production of ventricular fibrillation.[17, 18] That it did not occur in this series is perhaps due to the addition of the hypotensive technique.[12, 13] Hemostasis must be rigidly observed in the hypotensive-hypothermic patient, otherwise postoperative bleeding will result when the patient's blood pressure returns to a normal level postoperatively.[29] Postoperative hemorrhage has not been a complication in this series.

Posthypothermia circulatory insufficiency has been noted in patients in other series. The factors which seem to be most prominent as possible causes are: peripheral vasodilatation during the warming which may result in increased tissue oxygen utilization; and possible adrenal insufficiency which may result from the lack of adrenal response to trauma during the hypothermia, perhaps augmented by renal sodium loss.[30] That particular complication has not been noted in this small group of patients. The relative youth of these patients may have been a factor in its prevention.

A decreased platelet count has also been noted during hypothermia.[31] In general, however, it returns to normal with the return of the patient to normothermia. Other coagulation problems have also been observed,[32, 33] but these similarly have not arisen in this group of patients.

Of tremendous importance are the two recognized physiologic effects of low temperatures: to kill and to prolong life.[34] This particular technique must be used with that thought always uppermost in mind. If improperly used, its dangers could greatly outweigh its advantages, particularly in the pregnant patient, where not one, but two lives are involved.

Summary

1. The definitive operative therapy of a small number of patients with acute toxemia of pregnancy has been carried out under a hypothermic-hypotensive technique.

2. This technique was employed in an effort to make the surgical procedure necessary for the delivery safer for the mother and the fetus.

3. An extremely pleasing but unpredicted result has been the excellent infant survival.

4. The results to date with this ancillary technique in the treatment of acute toxemia of pregnancy seem to merit further consideration of its use.

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Discussion

DR. MILTON L. McCALL, New Orleans, La.—The greatest dangers of hypothermia are ventricular fibrillation, shivering, and a hemorrhagic diathesis which, according to Swan, is apt to occur in the postoperative period in the presence of rising blood pressure and lowered platelet count.

Heavy sedation and a ganglion-blocking drug are used in an attempt to overcome these complications. From the obstetrical point of view, however, it is unfortunate that such large doses of sedatives must be used. Dr. Barter gave his patients 250 mg. of pentobarbital sodium intravenously, and 25 mg. of chlorpromazine intravenously, followed by 10 mg. doses from time to time; then Pentothal Sodium administration by the intravenous route was started early in the procedure and continued or used repeatedly. This is far more sedation than we give our most seriously ill convulsive eclamptic patients. In fact, because it

is our distinct impression that many patients are overtreated, especially with large doses of intravenously administered barbiturates, we have for a number of years utilized a continuing vasodilator infusion of Unitensen and Apresoline with only mild sedation. The results have been most gratifying. Prolonged coma from medication with its concomitant complications is no longer a problem.

In the past 4 years there have been approximately 40,000 deliveries on the Louisiana State University Services and over 7,000 patients in whom a diagnosis of toxemia of pregnancy has been made. The most severely ill 4 per cent, the patients with hypertensive crises or convulsions, have been treated with the method described. There have been no maternal deaths, although Krupp found that toxemia was the most common cause of maternal death in Charity Hospital at New Orleans over a period of 10 years and that 47 per cent of these deaths were associated with gross cerebral hemorrhage. The perinatal mortality has been surprisingly low. During a recent period of 15 months, there were over 2,200 cases of toxemia of which only the 75 most severe were treated with vasodilator infusion. Of the 77 babies born, 71 weighed over 1,000 grams and 5 of these died. None of the mothers of these babies had had previous prenatal care, and in 3 of the 5 cases intrauterine death had already taken place at the time of admission to the hospital. Thus it is evident that comparatively simple management may accomplish at least some of our most important objectives in severe toxemia.

It also appears to be unfortunate that the antitensive agent used in conjunction with hypothermia is a ganglion-blocking agent. Assali has shown that such drugs do not affect the vasospasm of toxemia of pregnancy nearly as completely as certain other agents inasmuch as the vasospasm of this disease is apparently due more to humoral activity than to neurogenic influences. There are also special dangers which may occur in the pregnant patient. Vital adaptive reflexes are blocked out which may make both the mother and the baby quite rigid neurologically. Several authors, including Morris, have reported severe adynamic ileus and bladder paralysis as well as instances of rupture of the bladder and bowel in infants due to the paralyzing influences of ganglion-blocking agents given to their mothers.

While there are a number of obvious drawbacks to the use of hypothermia as it is presently practiced in pregnancy toxemia, in the future comparatively simple modifications may greatly enhance its value. Could other vasodilators, such as Unitensen-Apresoline blend, be utilized safely? If so, could less sedation be used? Even without marked modification this technique may find its greatest use in the severely ill patient who continues to have convulsions, or it may be indicated in the eclamptic patient who has been greatly depressed with too huge doses of barbiturates given intravenously.

DR. ALBERT.—We are quite aware that the eclamptic patient suffers from a process of vasoconstriction, and that cooling also produces vasoconstriction: this would naturally accentuate the existing state. In order to combat the marked degree of vascular spasm, moderate sedation and minimal doses of chlorpromazine were given which did not produce blood pressure changes. Controlled vasodilatation was then instituted by infusion of a peripheral blocking agent—Arfonad Camphorsulfonate.

Hypothermia depresses the metabolic and oxygen requirements of the central nervous system, thus protecting the vital centers against sudden changes in blood flow and oxygen delivery. Previous studies with hypothermia have shown the stress reaction to be delayed, giving extra protection to the organism as a whole.

The infant seems to benefit by the reduced temperature, which probably enables it to tolerate better the effect of depressant drugs.

Although the number of cases in this series is small, it has permitted us to study certain changes and reactions that occurred.

Sedation seems to be essential in order to produce the desired degree of vasodilatation with Arfonad infusion. The purpose of sedation is to depress the central mechanism controlling endogenous epinephrine discharge. Once vasodilatation has been produced by the combined effect of sedation and peripheral blocking agents, cooling proceeds rapidly with no untoward effects.

DR. THADDEUS L. MONTGOMERY, Philadelphia, Pa.—This paper has interesting implications regarding hypothermia as a means of fetal salvage. I am not sure that the results in the mother are superior to those which are obtained by more conservative methods of anesthesia and treatment in toxemia of pregnancy and delivery.

The survival rate of the fetus, particularly in the instance of premature fetuses and especially where such large dosage of sedation was given to accomplish appropriate anesthesia levels, suggests that hypothermia may prove to be an important factor in preserving fetal life when depression of function is present from disease, hypoxia, or analgesic and anesthetic drugs.

It may be that in the cases reported the hypothermic state in the fetus has acted as a protective phenomenon not only in regard to toxemia but also to the large doses of sedation and the depression of fetal respiration. Time will have to tell, however, whether these surviving infants were normal or impaired organisms. Certainly there is nothing in the data at the moment to indicate that the newborn was impaired by the experience.

DR. DUNCAN E. REID, Boston, Mass.—This is a most important paper because it has made us aware that perhaps when there is greater concern for fetal survival there might be greater hope by the use of this type of anesthesia. I doubt that the marked drop in respiratory rate in these babies was due to the drugs. I assume that it was due to the fact that the baby's body temperature was low and oxygen requirements were lessened.

I should like to ask Dr. Barter how long the respiratory rates remained low and whether he believes this is truly a result of drop in the body temperature rather than the effect of the drugs.

DR. BARTER (Closing).—Dr. McCall and I are in close agreement on this subject. There were 1,144 patients with toxemia in this series. Obviously we treated very few of the toxic patients with hypothermia. Our treatment of the average patient is very closely comparable to that of Dr. McCall's group in New Orleans.

In regard to the points brought out by Dr. Montgomery and Dr. Reid, we think the depression in the fetal respiratory rate is primarily due to the generalized depression of the baby by the cold. Actually it is in a temporary state of hibernation. All of the infants breathed spontaneously. There was no depression in the strict sense of the word. All had excellent color and all were perfectly normal.

There are specific reasons for not warming the infant. While it gradually assumes its normal body temperature of 37° C. it gets a chance to detoxify any barbiturate which it has acquired. The baby's respiratory rate increases gradually over several hours to a normal rate as it resumes its normal temperature. It is most important not to warm the baby from the standpoint that it is in a state of hypometabolism when it is chilled. If it is warmed too rapidly the full effect of the depressing influence of the agents which it has acquired through the placenta is imposed upon the infant.

IRRADIATION SENSITIVITY OF CERVIX CANCER*†

Response of Cultured Cervix Cancer Cells to Irradiation

NORMAN F. MILLER, M.D., PETER P. LUDOVICI, PH.D.,
ROBERT T. CHRISTIAN, PH.D., AND GARDNER M. RILEY, PH.D.,
ANN ARBOR, MICH.

(From the Reuben Peterson Memorial Research Laboratory, Department of Obstetrics and Gynecology, The University of Michigan)

CHEMOTHERAPY may some day become an important weapon in the treatment of cervix cancer. For the present, however, and perhaps for many years to come, we shall continue to depend on surgery and irradiation for this purpose. While surgery has demonstrated its usefulness in some cases, our principal therapeutic weapon continues to be irradiation. Because this is so, a closer look at what we are doing is in order. Many aspects of contemporary irradiation therapy are the result of painstaking trial and error study. Much effort has been devoted toward standardizing treatment. While the proclaimed end point is destruction of the neoplasm, treatment has, in fact, been largely determined by the behavior of normal tissue. Generally speaking, in treating with irradiation the tendency is to administer a dose based on what normal tissue will tolerate, and if by so doing the cancer is destroyed everyone is happy. Yet some cancers may be destroyed by less than the standard amount of treatment, while others may require more for their destruction. If we were able to single out the irradiation-resistant neoplasms we might then resort directly to radical surgery. There is nothing new about this type of thinking. Pathologists have tried for years to tell us what might be expected on the basis of histologic cancer grading. So, too, Ruth Graham[1, 2] has described a cytologic method, based on the evaluation of benign vaginal cells, which is considered useful in selecting the irradiation-responsive patient. A specific change in the *benign* basal cell before treatment, known as the sensitization response, or SR, is reported accurate in predicting whether the cancer will be controlled by x-ray treatment. Graham further suggests that the effectiveness of irradiation therapy can be gauged by specific cellular changes, the so-called radiation response, or RR, which occurs in *benign* squamous cells seen in the vaginal smear either during or immediately after irradiation treatment. Although 5 year survival studies suggest that both methods are of prognostic value, the significance of SR and RR cells requires further investigation and substantiation.

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†Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

Glücksmann[3, 4] reported on a quantitative histologic method of assessing the irradiation response of cervix carcinoma. Using serial tumor biopsies during treatment, he found that an unfavorable response was characterized by persistence of mitotic activity and little or no alteration in the number of differentiating cells. A favorable response on the other hand, was characterized by early disappearance of mitotic and resting cells, and an increase in the number of differentiating and degenerating cells. This histologic assessment technique was found to have some value in prognosis. Because of the need for obtaining successive representative biopsies, however, it has not yet developed into a practical procedure. Gusberg and co-workers[5] have studied nucleoprotein patterns by special staining techniques in an endeavor to determine the radiosensitivity of tumor cells.

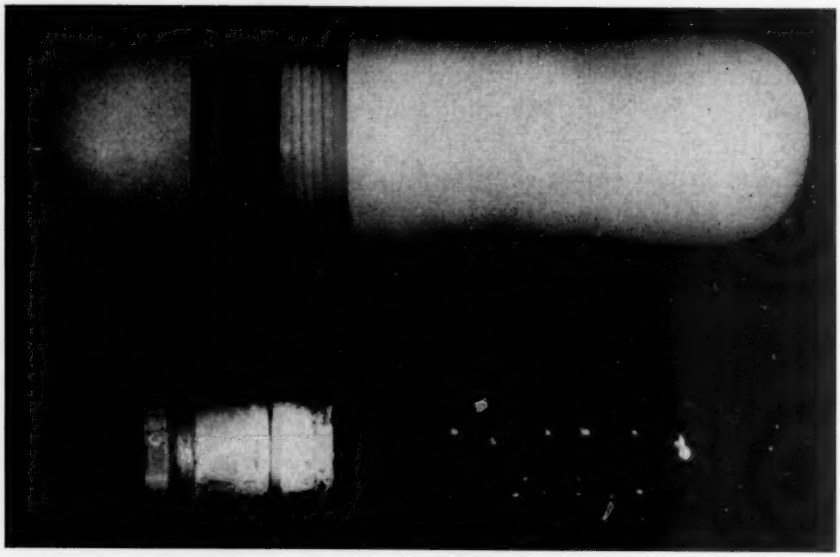

Fig. 1.—Nylon plastic screw-cap container and small roller tube culture used for the in situ clinical irradiation (Series I).

The feasibility of a tissue culture approach to the study of tumor response to irradiation is indicated by the fundamental studies of Spear and his collaborators[6, 7, 8] at Strangeways Laboratory in England. Spear's basic work using cultured animal tissue demonstrates that the biologic effects of small doses of irradiation are similar whether the *normal* cells are irradiated in vitro (tissue culture) or in vivo. Recently, Lasnitzki[9] has compared the in vitro and in vivo response of malignant animal cells to irradiation. She found that the direct effect of small doses of irradiation was similar in either case.

This report describes a new in vitro approach to the problem of predicting the radiation sensitivity of *human* tumors.

Materials and Methods

Tissue Culture.—Preirradiation biopsy specimens of tumor tissue were obtained from each patient and suspended in a depression slide containing a

mixture of equal parts human placental serum, penicillin (10,000 units per milliliter), and streptomycin (10 mg. per milliliter). After one half hour the mixture was replaced with fresh human placental serum and the tissue cut up into small fragments approximately 1 c. mm. in size. Two types of roller tube cultures were employed. One was 50 mm. in length and 16 mm. in diameter (Fig. 1) used primarily for the in situ clinical studies (Series I), whereas the other was the standard 150 mm. in length and 16 mm. in diameter test tube (Fig. 2) used for the pelvic phantom work (Series II). Approximately 25 explants for the smaller tubes and 40 explants for the larger ones were placed directly on the inside glass surface by means of a bent-tipped capillary pipette.[10] After one half hour the transferral fluid consisting of human placental serum had drained to the bottom of the tube and was replaced with exactly 0.35 ml. of nutrient fluid. The composition of the standard nutrient fluid was 2 per cent chick embryo extract, 58 per cent Hanks balanced salt solution, and 40 per cent human placental serum. Penicillin and streptomycin were also incorporated at a final concentration of 100 units and 0.1 mg. per milliliter, respectively. The tubes were capped and then incubated at 37° C. in a roller drum apparatus designed to complete 12 revolutions per hour. The medium was usually changed daily after microscopic examinations of the cultures.

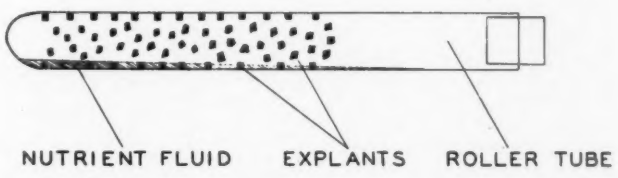

Fig. 2.—Illustration of a standard roller tube culture used for the pelvic phantom irradiation (Series II).

In Situ Clinical Studies.—Series I experiments were carried out by an in situ clinical procedure. This consisted of irradiating the culture for a given patient at the same time she received her therapeutic treatment. For this purpose the patient's cultured cells were placed in a specially constructed nylon plastic screw-cap container (Fig. 1) and inserted into the vagina adjacent to the cervix. Immediately after treatment the culture was returned to the incubator. For direct comparison, a nonirradiated control was also carried for each patient.

Clinical x-ray therapy was carried out with a unit having a half-value layer of 1.5 mm. of Cu, field sizes of 15 by 12 cm. at a target-skin distance (T.S.D.) of 50 cm. in an air dose rate of 32 r per minute. The therapeutic cobalt^{60} unit had a field size of 11 by 8 cm. and 11 by 6 cm., with a dose rate of 52 to 58 r per minute.

The patients were irradiated by the standard 4 port method. Two anterior ports on one day were alternated with two posterior ports the next day. The calculated midline dose for patients receiving cobalt^{60} therapy was used to estimate the amount of irradiation received by the cultured cells.

Exact midline doses could not be calculated for the x-ray-treated patients. For this reason, actual measurements* of the doses received by the cultured

*We are indebted to Dr. Charles S. Simmons and his staff of the Department of Radiology for these measurements as well as those made in the pelvic phantom. We would also like to express our gratitude for his assistance in supervising the construction of the pelvic phantom as well as his technical assistance in the interpretation of irradiation-dosage data.

cells within the tubes were made with a dosimeter on 4 of the patients during 2 consecutive days of treatment. On the basis of these data, significant estimates of the dose to the tissue culture tube could be made on the remaining x-ray-treated patients.

Pelvic Phantom Method.—Experiments in Series II were conducted in a pelvic phantom. This consisted of irradiating culture tubes in a pelvic masonite phantom (Fig 3) under conditions simulating those received by the patient's tumor cells in situ. Each culture was exposed to a daily air dose approximating that received by the patient.

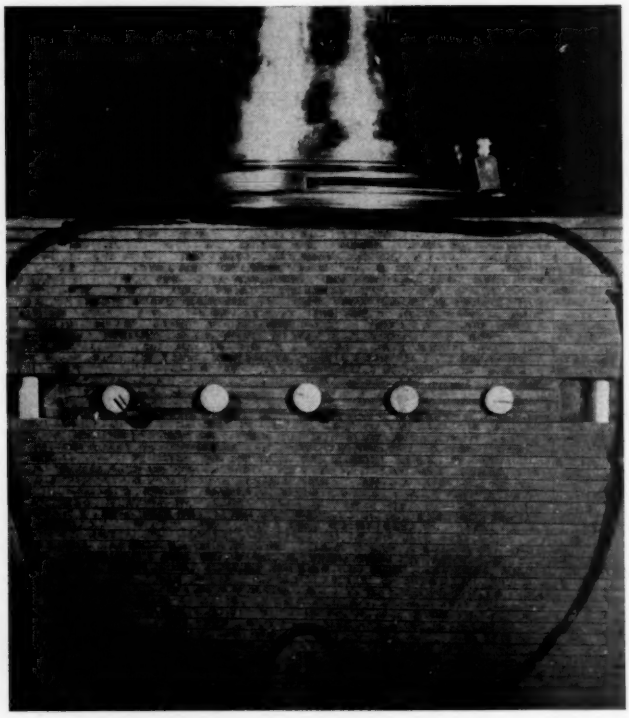

Fig. 3.—Roller tube preparations positioned in adjustable pelvic phantom beneath x-ray cone.

Irradiation was carried out with a unit having a half-value layer of 1.3 mm. Cu, field size 15 cm. diameter at a Target-skin distance (T.S.D.) of 50 cm., and an air dose rate of 28 r per minute. The actual dose to the tissue for each position in the phantom was measured with a Baldwin dosimeter. In order to duplicate the dosage as closely as possible, the dosimeter readings were made with the probe inserted in a glass culture tube. Readings were corrected to standard temperature and pressure.

Evaluation of Irradiation Effects.—The response of cultured cells to irradiation was determined by daily cytologic observations with an A. O. Spencer microscope with the use of 2.5 and 10× objectives and recorded by means of 35 mm. photography. The criterion selected for the end point of irradiation effect was the destruction of *all* outgrowing tumor cells. Certain cultures ceased growing after partial irradiation only to manifest regrowth some weeks later. For this reason all cultures received full irradiation and were maintained for at least one month after all growth had ceased.

Results

Growth Characteristics.—The roller tube method described permits good growth of most cervix carcinomas as well as other female genital cancers. The majority showed an outgrowth within 2 days. Some produced growth as early as 6 hours after explanation and a few did not produce tumor cells until the fourteenth day. Because the carcinoma cell type is epithelial in nature, it is usually distinguishable from contaminating stromal fibroblasts. Since, in most cases, the fibroblasts did not appear until the twelfth to the fourteenth day of cultivation, the carcinoma cells grew out and became well established before the fibroblasts made their appearance. In the few instances where the epithelial cells appeared late, the explants first put out an extensive reticulum of fibroblastic elements (4 to 5 days). These spindle cells were then pushed away from the explants by a compact epithelial sheet of carcinoma. Fibroblastic elements in these cases were not of the aggressive type; that is, they showed no tendency to infiltrate and destroy the epithelial sheet. On the other hand, late-growing fibroblastic elements in some cases appeared to be extremely aggressive, rapidly infiltrating, outgrowing, and finally destroying the carcinoma sheet. Another type of fibroblastic growth noted in a few cases was the formation of a protective-like capsule completely surrounding the carcinoma cell sheets. The significance of these different types of fibroblastic outgrowths and their role in cervix malignancy is being investigated further.

Different types of epithelial outgrowths were observed. In some instances only compact sheets of cells would emerge, while in others individual, loose epithelial cells appeared. Apparently these cells were unable to form the intercellular bridges which permit the compact adhesiveness noted with the sheet-forming types. These loose cells had a tendency to round up and varied considerably in size from small basal types to large squamous forms. In other cases a mixture of compact adhesive sheets and individual loose cells was observed growing out from the same explant.

The adenocarcinomas of the cervix were indistinguishable from the squamous-cell carcinomas during the first 4 days of cultivation. Thereafter the cells usually differentiated to form typical glandlike spaces seen in histologic sections of these tumors.

Vaginal carcinomas usually grew in compact epithelial sheet patterns consisting of typically large, adult squamous cells.

Cultures were exposed to the first irradiation treatment when a majority of the explants had well-established outgrowths. The first exposure was generally between the fifth and the seventh day of cultivation. In rare instances, when the epithelial outgrowth did not occur until the fourteenth day, exposure to irradiation was delayed until the sixteenth to the twenty-first day of cultivation. These cases could be included in the pelvic phantom series (II), but had to be omitted from the in situ series (I) because the patients' clinical therapy was already under way by this time.

In Situ Series.—Tissue from 25 patients was studied by the in situ method. For one reason or another to be mentioned later, in only 10 of the 25 cases were studies completed satisfactorily. Of these, 8 were squamous-cell cervix carcinomas, one was an adenocarcinoma of the cervix, and the other, a carcinoma of the vagina.

The results of the in situ irradiation effects are presented in Table I. It is evident that there are definite differences in the in vitro irradiation sensitivity of these tumor cells. A correct mathematical formula which would permit a grading of these differences cannot yet be developed. When the 5

year survival rates for these patients are available, it may be possible to formulate an equation which will permit a positive correlation between the in vitro tissue irradiation sensitivity and the in vivo therapeutic response.

Meanwhile, a tentative method for comparing the results is proposed. We have chosen to express the differences in radiation effect by the simple formula, $E = \frac{r \times S}{1{,}000}$, where E is the tissue culture radiation effect index, r the lethal or total roentgen dose, and S the number of days the culture survived subsequent to the first day of irradiation. For example, if the cultured cells received a total of 1,000 r and survived for 75 days, then $E = \frac{1{,}000 \times 75}{1{,}000}$, or $E = 75$. We have tentatively assumed that values of 0 to 49 represent irradiation-sensitive tumors; 50 to 99 represent tumors with an intermediate irradiation sensitivity, and 100 or greater represent relatively irradiation-resistant cancers.

On this basis, in our Series I, of 10 cases, we have classified 3 as resistant, one as intermediate, and 6 as irradiation sensitive. The nonirradiated control cultures of the 3 resistant, one intermediate, and 6 sensitive cases averaged, respectively, 16, 20, and 13 days longer survival than their irradiated counterparts. Furthermore, these control cultures consistently showed greater metabolic activity as evidenced by increased pH depression than their corresponding irradiated cultures.

TABLE I. INTRAVAGINAL IRRADIATION OF TISSUE CULTURED GENITAL CANCER* (SERIES I)

CASE NO.		TISSUE SOURCE	DOSE† IN R	SURVIVAL (S) DAYS‡	PROBABLE SENSITIVITY EFFECT INDEX§	CLASSIFIED
1	180	Carcinoma of cervix	2,484‖	93	231	Resistant
2	269	Carcinoma of vagina	4,250¶	44	187	Resistant
3	293	Carcinoma of cervix	1,716	97	166	Resistant
4	266	Carcinoma of cervix	2,333	34	79	Intermediate
5	239	Carcinoma of cervix	1,913	19	36	Sensitive
6	278	Carcinoma of cervix	942	21	20	Sensitive
7	262	Carcinoma of cervix	1,280	14	18	Sensitive
8	246	Carcinoma of cervix	1,238	13	16	Sensitive
9	214	Carcinoma of cervix	623¶	21	13	Sensitive
10	182	Adenocarcinoma of cervix	585	7	4	Sensitive

*Nonirradiated control cultures survived longer than corresponding irradiated cultures.
†Actual lethal or total roentgen tissue dose.
‡Total days the culture survived subsequent to the first day of irradiation.
§Effect index equals $\frac{r \times S}{1{,}000}$, where r is the lethal or total roentgen dose, S the number of days the culture survived subsequent to the first day of irradiation.
‖1,170 r x-ray and 1,314 r cobalt^{60}.
¶Cobalt^{60} therapy.

Present survival data indicate that Case 1 (Table I) died 5 months after treatment and Case 2 (Table I) developed a recurrence in 9 months. Both of these were irradiation resistant. The remaining 7 cases have shown a clinically satisfactory response ranging from 6 to 21 months.

Pelvic Phantom Series (Series II).—This series includes 9 squamous-cell carcinomas and 5 adenocarcinomas of the cervix. The results for the former are presented in Table II. Here again we note differences in the in vitro irradiation sensitivity of these tumors. Classification according to the method described above suggests that 5 were resistant, one was intermediate, and 3 were irradiation sensitive. A typical set of photographs illustrating the effect noted is presented in Figs. 4 and 5.

TABLE II. PELVIC PHANTOM IRRADIATION OF SQUAMOUS-CELL CERVIX CARCINOMAS* (SERIES II)

CASE NO.	DOSE† IN R	SURVIVAL (S) DAYS‡	PROBABLE SENSITIVITY	
			EFFECT INDEX§	CLASSIFIED
1 315	4,937	80	394	Resistant
2 341	3,980	60	239	Resistant
3 343	4,800	46	211	Resistant
4 330	4,380	29	127	Resistant
5 339	3,780	30	113	Resistant
6 321	2,834	22	62	Intermediate
7 335	2,268	16	36	Sensitive
8 327	2,628	12	32	Sensitive
9 336	1,440	7	10	Sensitive

*Nonirradiated control cultures survived longer than corresponding irradiated cultures.
†Actual lethal or total roentgen tissue dose.
‡Total days the culture survived subsequent to the first day of irradiation.
§Effect index equals $\frac{r \times S}{1,000}$, where r is the lethal or total roentgen dose, S the number of days the culture survived subsequent to the first day of irradiation.

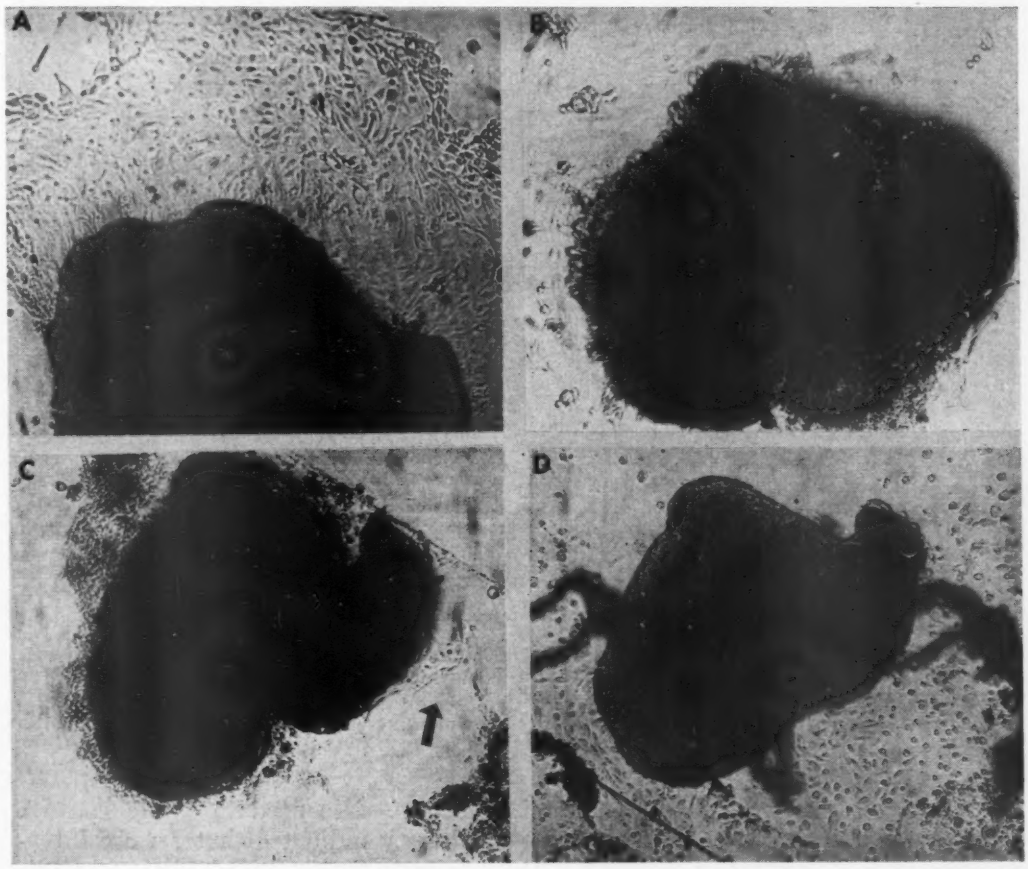

Fig. 4.—Typical effect of irradiation on one explant from a probable resistant type squamous cell tumor (Case 2, Table II). *A*, Preirradiation. *B*, Seven days after the first treatment showing initial disappearance of outgrowth after 1,939 r. *C*, Thirty days after the first treatment showing the reappearance of tumor cells (arrow) after a total dose of 3,980 r. *D*, Fifty-six days after the first treatment showing the extensive regrowth of neoplastic cells after a total dose of 3,980 r. (Approximately ×75; reduced ⅜.)

The nonirradiated control cultures of the resistant, intermediate, and sensitive squamous-cell groups averaged, respectively, 11, 38, and 16 days, longer survival than their corresponding irradiated cultures. Doubtless the control cultures would survive much longer were it not for the proliferation of fibroblastic elements which eventually overgrow and destroy the tumor cells. In the irradiated cultures, on the other hand, this is not a serious problem, since irradiation is effective in slowing down the growth of fibroblasts to a point where they do not interfere with the tumor cells.

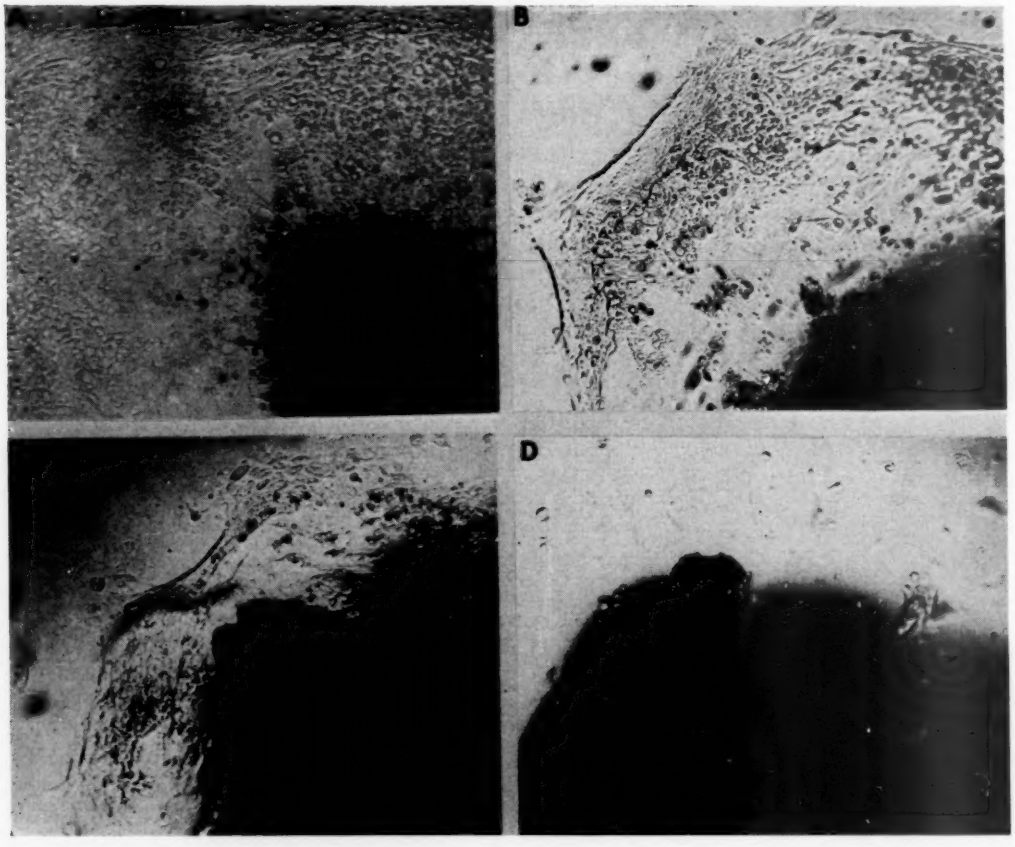

Fig. 5.—Typical effect of irradiation on one explant from a probable sensitive-type adenocarcinoma (Case 10, Table I). *A*, Preirradiation showing an extensive adenocarcinoma sheet completely surrounded by a protective fibroblast-like capsule at the periphery. *B*, Three days after the first treatment showing the shrinkage of the epithelial sheet and the thickening of the fibroblast-like capsule after 351 r. *C*, Four days after the first treatment showing further shrinkage after 468 r. *D*, Five days after the first treatment showing complete disappearance of the epithelial outgrowth after 585 r. (Approximately ×75; reduced ⅜.)

The data for 5 cervix adenocarcinomas are presented in Table III. A tentative classification of their relative sensitivity indicates that 2 were intermediate, and 3 irradiation sensitive. It is of interest that up to now no irradiation-resistant adenocarcinomas of the cervix have been found. The significance of this observation cannot be evaluated until a larger number of cervix adenocarcinomas have been studied. Nonirradiated controls of the intermediate and sensitive adenocarcinoma groups averaged, respectively, 28 and 33 days' longer survival than their irradiated counterparts.

TABLE III. PELVIC PHANTOM IRRADIATION OF CERVIX ADENOCARCINOMAS* (SERIES II)

CASE NO.	DOSE† IN R	SURVIVAL (S) DAYS‡	PROBABLE SENSITIVITY	
			EFFECT INDEX§	CLASSIFIED
1 322	3,648	19	69	Intermediate
2 344	3,824	17	65	Intermediate
3 340	3,120	14	44	Sensitive
4 329	2,640	11	29	Sensitive
5 324	1,477	7	10	Sensitive

*Nonirradiated control cultures survived longer than corresponding irradiated cultures.
†Actual lethal or total roentgen tissue dose.
‡Total days the culture survived subsequent to the first day of irradiation.
§Effect index equals $\frac{r \times S}{1,000}$, where r is the lethal or total roentgen dose, S the number of days the culture survived subsequent to the first day of irradiation.

Comment

The data presented in this report indicate that some tissue cultured cervix carcinoma cells are resistant to irradiation, whereas others are readily destroyed. This was true whether the in situ or simulated clinical irradiation technique was used.

As might be expected, the phantom technique has many advantages over the in situ (Series I) method. Thus, not all patients are suitable candidates for the in situ method. Anatomic variations, differences in temperament and mental outlook, as well as the clinical treatment schedule, render this method undesirable. The phantom technique has none of these drawbacks. It permits rigid standardization of both culture and irradiation conditions. Furthermore, reproducibility can be investigated by exposing duplicate tubes to irradiation. Studies of this nature are now in progress and will be reported in the future.

It should be pointed out that success in growing human carcinoma depends a great deal on the number of explants cultivated per tube. The more tissue cultivated, the better is the chance for explanting viable tumor fragments and, perhaps more important, there must be abundant tissue present per tube to initiate growth. Apparently, tissue breakdown products are needed for conditioning the medium so that it will be capable of supporting growth.

As mentioned previously, the primary purpose of this study was to determine the relative irradiation sensitivity of patients' cultured cells with the thought that the in vitro index of behavior might reflect the tumor's irradiation response in vivo. The problem, of course, is a complex one. We recognize that other factors such as the clinical extent or stage of the disease, as well as host resistance, play important roles in prognosis. While the clinical extent of the neoplasm can be approximated, we have as yet no satisfactory method for the determination of host resistance. It remains to be seen whether host resistance will loom as a more important factor than tumor irradiation response.

It must be emphasized that this report represents a preliminary effort to devise an in vitro technique for determining the irradiation sensitivity of

human cervix carcinoma. It is still premature to assume that the difference in irradiation sensitivity of tumor cells observed in vitro truly reflects their irradiation response in vivo.

Summary

1. A new in vitro approach to the problem of predicting irradiation sensitivity of female genital tumors is described.

2. The results indicate that some carcinomas are relatively resistant to irradiation, whereas others are readily destroyed. Although the results appear promising, it is premature to assume that irradiation sensitivity of cultured cancer cells in vitro truly reflects the tumor's response in vivo.

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Discussion

DR. GEORGE A. HAHN, Philadelphia, Pa.—Tissue cultures both provide nutrition for the growth of cells and also serve as a framework for cell migration. The culture method differs significantly from heterotransplants in which the anterior chamber of the eye in rabbits and guinea pigs or the cheek pouch of the hamster may be used for the growth of human tumors. Here one must deal with genetic factors inherent in the animal strain itself, and alterations due to the age, sex, hormonal status, and general health of the animal being used.

The transplantable tumor may elicit host defense reactions that do not ordinarily occur with spontaneous malignancy but yet will change the behavior of the transplant.

Southam has been using human volunteers and has noted the increased receptivity of the volunteer as a host if cancer is already present elsewhere in the body.

Pure tumors grown in tissue culture usually require larger amounts of x-ray irradiation for their destruction than are necessary to prevent growth of similar tumors in animals.

The amount of vascularization of the tumor and the tumor bed itself, and the size and location of the tumor may serve to alter significantly the clinical result of x-ray therapy, despite a favorable biologic response of the malignant cells to irradiation.

There is apparently a difference in the ease with which different tumor explants adapt themselves to tissue culture. Is this adaptability a measure of malignancy? Are cervix tumors made up of varying types of cells, only some of which are capable of tissue culture?

It is evident that elements other than the malignant cells alone must be considered for a complete understanding of the carcinocidal effects of irradiation.

Cobalt^{60} teletherapy was used as a means of treatment in a proportion of the cases. Were comparable cases and control cultures used so that it was possible to arrive at an evaluation of the relative biologic effectiveness of cobalt and x-ray therapy?

Radiosensitivity within a therapeutic dosage range depends upon factors inherent in the cells and cannot be indefinitely increased by progressive change in irradiation.

This paper arouses much speculation in my mind. By the in vitro tissue culture of cells from the patient's tumor, it may be possible to improve our results in irradiation therapy by greater individualization of treatment, perhaps changing time and dose relationship, depending on the cellular response to therapeutic agents. The addition of chemical agents, changes in oxygen tension, and other types of irradiation (neutron) may in combination improve our survival rates.

Dr. Miller has presented a new in vitro method for determining the radiation sensitivity of human tumors. In a way it may be called a biologic ionization chamber. The mathematical formula suggested gives a means of prognosticating the irradiation response of human tumors.

DR. AXEL N. ARNESON, St. Louis, Mo.—Physical factors that affect depth dose and distribution of radiation have been extensively explored. Knowledge of biologic response of tissues is less advanced, and researches in that area present the most probable means for improving end results in cancer treatment. I believe that to be the important significance of the experiments reported by Dr. Miller and his coauthors. Any success they attain in establishing a reliable test of radiosensitivity will be a by-product of their more basic investigations.

In selecting an in vitro technique of study, Dr. Miller takes cognizance of the absence of host influence in assessing the effects of irradiation. There is evidence that tumor regression is the result of direct trauma to cancer cells, as well as complex biophysical changes induced in the tumor bed. We do not know the relative importance of each, but it can be said that the recognizable changes in the tumor bed are essentially fibrosis and ischemia. Despite the fact that Dr. Miller's experiments are conducted in vitro, it is most interesting to note that the observed changes in cancer explants simulate the histologic alterations found in serial biopsy taken from patients during external irradiation preceding the use of radium.

The most striking similarity is the regrowth of tumor after partial irradiation. We have also found evidence of recovery after initial degenerative changes in a few patients treated experimentally with very small doses over an unusually protracted period, despite the fact that the tumor continued to be exposed to small amounts of radiation. It is believed that explanation of those phenomena is to be found in the dose-time relationship. The data reported by Dr. Miller and his co-workers suggested that cancer cell recovery from radiation effects at the subtolerance level may be independent of host influence.

There are two other points that should be mentioned but each is considerably more vague than the first. Among the several photomicrographs, Dr. Miller illustrates the effect upon an explant from a probable resistant type of squamous-cell cancer. I wonder if there are not detectable differences in the cellular structure of the residual growth as compared with the unirradiated specimen? In clinical practice one often finds that residual cancer presents microscopic evidence of maturation over that noted in the original diagnostic biopsy. I should like to ask Dr. Miller if he has noted any differences in appearance in the residual growths in explants and, if so, does he consider that an example of in vitro effects simulating in vivo changes? The last comment is in relation to the author's observations upon fibroblasts. Those late in development sometimes appeared more aggressive than the earlier-appearing forms. With aggressive action there was invasion and extinction of cancer cells. It is not stated whether such action has any relationship to radiation exposure but the phenomenon simulates certain responses of normal host tissues to irradiation. Can Dr. Miller assign this phenomenon to any type of environmental response?

Finally, I must return to the author's primary objective: a test of radiosensitivity. In commenting upon that, however, I should like to include the in vitro and in vivo aspects. Present data are largely preliminary, but even so the number of "sensitive" explants appears low in comparison with clinical experience. The lower ratio of sensitive

explants may reflect the absence of host influence. Thus, in my opinion, the entire project revolves upon basic research. Dr. Miller and his associates set out to study a problem with immediate clinical application. I predict that further exploration in their work will soon encounter complex and provocative problems of great appeal. Here is an example of clinical leadership in the basic sciences. I believe that to be important. Dr. Miller has mentioned the standardization in reference to tolerance of normal tissues that may be applied to radiotherapy. The radiologist, as well as the gynecologist, too often attempts to treat all patients to the so-called limits of tolerance. They sometimes appear obsessed with the aim to deliver certain predetermined doses to specified points within the pelvis. Are they thus not guilty of treating imaginary points rather than the patient? Nolan and others have presented convincing evidence that early cases do better if undertreated in terms of dose applied to more advanced forms. In working directly with the tumor cell, Dr. Miller and his coauthors will extend our knowledge of biologic response.

DR. MILLER (Closing).—Dr. Hahn asked if we might be getting a selected type of growth, that perhaps only certain types of cells might be growing out from the explants. I believe this is possible, but it is also true that the cells that do grow out are probably the most malignant or active cells in the entire explant specimen. We know it is difficult to grow normal tissue, whereas neoplastic tissue grows readily, so in our testing we are probably dealing with the more active cells.

Dr. Arneson asked whether we noted maturation of the cells in culture after their exposure to irradiation, and this we cannot answer. It is something we will look into.

He also asked about fibroblastic proliferation. I did not mention it in my talk but this point is discussed in our paper so I will not take time now to comment upon it. Many other questions have been asked which I shall not endeavor to answer here. Some will be discussed in the paper whereas others are not yet answerable.

Dr. Hertig handed me two questions which I shall read: (1) "Did you use the effect of the tissue culture either in the vagina or phantom to gauge or determine clinical dosage?" This, of course, is the aim and purpose of our study. If we can correlate tissue culture findings with patient survival rates we may be able to use this technique as a tool to determine dosage.

(2) "Was there histologic study on the explants to determine whether they were radiosensitive or resistant?" We have taken explanted material and subjected it to histologic evaluation but the bits of tissue are small and so far we have inadequate histologic data of this sort to warrant comment on this point. A good deal more needs to be done, including cytologic study and evaluation of the important host resistance factors before some questions can be answered.

EARLY DIAGNOSIS AND TREATMENT IN A CANCER SURVEY PROGRAM*

Summary of a Two-Year Project

JOHN C. ULLERY, M.D., AND
ZEPH J. R. HOLLENBECK, M.D., COLUMBUS, OHIO

(From the Department of Obstetrics and Gynecology, College of Medicine, The Ohio State University)

THE scope of cytological examinations of various parts of the human body has been increased markedly in the past few years. This is particularly true of cancer of the female pelvis. The accessibility of the pelvic organs and the exfoliation from this area offer a singularly adaptable method for the study of altered cells suggesting the presence of malignancy.

In a preliminary report made one year ago, we indicated our experience in this survey with the detection of 99 patients with pelvic carcinoma. The purpose of this presentation is to show the results of the 2 year study. At this time conclusions of greater value can be made.

The Cancer Survey Program of Columbus, Ohio (and Franklin County, Ohio), has been sponsored by the National Institutes of Health, Bethesda, Maryland, and the Department of Pathology and the Department of Obstetrics and Gynecology of the College of Medicine, The Ohio State University.

Perhaps in some, little enthusiasm will be generated for the early detection of cancer of the cervix with the necessity for adequate treatment by the figures of its frequency often quoted in medical journals. However, when other surveys now in progress[2-6] show that the presence of uterine cancer is revealed in 0.4 to 0.8 per cent of the female population screened, the problem becomes a serious one. Additional emphasis must be placed on this disease when we consider that in all types of cancer of the cervix, the 5 year survival rate is less than 40 per cent.

The cancer survey program has steadily maintained its three objectives during these 2 years: (1) to assess the value and feasibility of the use of exfoliative cytology methods in the early detection of pelvic cancer; (2) to use these methods to screen as large a number as possible of the female population of age 20 and over in Columbus and Franklin County; and (3) to correlate and evaluate the treatment given to patients in whom cancer was found.

*Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

The program is carried out primarily along lines similar to the Memphis Plan reported by Erickson and his group.[4] The examinations are performed on all women of 20 years or older by a single vaginal aspiration and repeated at least 3 times at intervals of one year. The presence of any suspicious malignant cells immediately leads to further studies of the tissue to determine the value and efficiency of the cytological method. These consist of scraping smear, punch, cold-knife conizing biopsy, or fractional curettage, and are essential for final definite diagnosis.

A Central Coordinating Committee was first established, consisting of a pathologist, the director of the Columbus Cancer Clinic, the president of the Columbus Academy of Medicine (county medical society), and chiefs of service of obstetrics and gynecology of the leading hospitals of Columbus. Endorsement was given by the Columbus Academy of Medicine to setting up clinics in various areas of the city, and enthusiastic support by physicians has been obtained.

The cytology laboratory required the training of technicians, secretaries, and a supervisor. This was done by Dr. Emmerich von Haam, Professor of Pathology, and preparations were made to process and report 1,000 examinations each week.

Inasmuch as the Memphis study consisted of vaginal aspiration, this technique has been followed in all patients. Studies could thus be obtained that would show comparisons of the incidence of uterine cancer in Columbus and that reported in Memphis. The vaginal aspiration method offers the widest use of a mass screening project because of the ease of obtaining smears. In addition, this technique allowed the use of trained technicians to obtain the specimens on clinic patients and thus freed physician manpower to handle the clinics.

Because of the close integration of the Department of Obstetrics and Gynecology of the College of Medicine at The Ohio State University with obstetricians and gynecologists of Columbus, it was planned that the 26 members of this staff would use the cervical scraping technique in addition to aspirations on all their private patients. This would serve as another study for comparison of the two techniques. It would also help to verify or deny Cuyler's[7] report that cervical scraped specimens yield a higher degree of accuracy than vaginal-pool examinations for the presence of invasive or intraepithelial carcinomas.

County- and city-wide publicity for the program was obtained by fine cooperation of the newspapers, radio, and television. A special color movie of 8 minutes' running time was prepared showing the ease and small amount of time the examination required. Emphasis was directed to interesting the well woman in having the examination for the prevention of uterine cancer and stressing the value of detecting early cancer before symptoms developed. A continuing program of lectures and discussions with the showing of the movie to various women's clubs and organizations has been in progress throughout the year.

The cytology laboratory receives all smears, which are prepared according to the Papanicolaou technique. The cytology examinations are done by trained technicians and physician cytologists. Special lettering is placed on each slide to differentiate them in the corollary investigations: *A* denotes aspiration, *S* cervical scraping.

The slide examinations are then reported to the clinic director or private physician according to this grouping: Group I, normal—negative; Group II, normal cells with some atypical cells—negative; Group III, severe dysplasia with suspicious cells present—suspicious; Group IV, many atypical and suspicious cells strongly suspicious of malignancy—positive; Group V, malignant, numerous malignant cells present—positive.

The clinic director or private physician then notifies the patient according to the grouping and interpretation. If results are normal, she is informed that the test is negative and told to return in one year, or earlier if any symptoms arise. Group II women are reported as negative but advised to repeat the examination, while Groups III, IV, and V call for the return of the patient and a complete tissue diagnosis. Any unsatisfactory slide examinations by the cytologist also call for repeat aspiration.

Upon the completion of 2 years on May 1, 1958, 90,786 slides had been received. The sources of these are noteworthy. As mentioned above, the program was initiated only after the endorsement and the cooperation of the county medical society had been obtained. The Central Coordinating Committee has believed from the very start of the program that the physician in private practice is the cornerstone upon which the success of the program rested. Accordingly, only patients who had no private physicians were given appointments and examined in the Central Cancer Clinic or other hospital clinics. All others were referred to their physicians. The success of our private-physician participation in the program is shown in Table I.

TABLE I. SLIDES RECEIVED

	NO.	%
Private physicians	66,392	73
Cancer survey clinic	8,108	9
Other clinics	16,286	18
Specimens received	90,786	100

The results of the cytological examinations of the cancer survey program are shown in Table II.

TABLE II. SLIDES REPORTED

	NO.	%
Negative reports	87,870	97.7
Unsatisfactory	1,245	1.4
Suspicious	872	0.9
Total	89,987	100.0

Of the 90,786 slides received in the 2 year period, 60,184 women received the aspiration examination or the aspiration-plus-smear examination. Two hundred and five patients were found to have cancer of the genitals as proved by subsequent tissue examination. One hundred and forty-five of these cases were detected by aspiration, 24 had a true false negative report, and 36 were in the missed false negative group, 14 of which occurred in the first 6 months of the survey.

This serves to emphasize that as our cytologists gained experience and training the frequency of false negative examinations markedly decreased.

Of the 18 slides in which malignant cells were overlooked, 4 patients had adenocarcinoma of the cervix or endometrium.

A questionnaire was sent to the physicians and clinic directors responsible for obtaining the initial examinations of the 205 patients in whom cancer was

detected, to determine whether or not cancer was suspected at the patient's initial visit. The number of patients with suspected and unsuspected cancer in this group and the source of these patients are shown in Table IV.

TABLE III. FALSE NEGATIVE REPORTS

Total number examinations	90,786
Cases of cancer detected	205
Suspected	145
True false negative	24
Missed false negative	36

TABLE IV. POSITIVE CASES AND THEIR SOURCE

	NO.	%
Suspected at First Visit.—		
Suspected clinically	42	20
Unsuspected	149	73
Not specified	14	7
Total	205	100
Source.—		
Private physicians	152	74
Cancer survey clinic	5	3
Other clinics	48	23
Total	205	100

In the 60,184 patients examined who had vaginal aspirations, cervical visualization and scraping were performed on 13,150 immediately following the aspiration. Eighteen patients with cancer were found on cervical-scraping smear examination who did not show malignant cells on aspiration (true false negatives). Ten of these were carcinoma in situ, and 8 were invasive. This serves to emphasize the value of the scraping test and substantiate Cuyler's[7] findings.

TABLE V. ANALYSIS OF ASPIRATIONS AND SMEARS

Total number examinations	89,987
Total number slides received	90,786
Aspiration only	90,786
Aspiration and smear	13,150 (Approx.)
Cancer suspected on smears that were negative on aspiration	18

The origin of the cancers found in the survey program are shown in Table VI.

TABLE VI. ORIGIN OF CANCERS IN 205 TOTAL POSITIVE CASES TRACED FROM MAY 1, 1956, TO MAY 1, 1958

Cervix—in situ (1 during pregnancy)	81
Cervix—invasive (3 in cervical stump)	94
Corpus	26
Vagina	3
Ovary	1
Total	205

The clinical staging of the invasive cancers of the cervix is shown in Table VII. This is based on the international classification. The figures are subject

to error, as it was necessary in some instances for us to go back to the private physician who had originally used the aspiration examination or had managed the treatment to obtain his original classification. In several instances it required close questioning to try to ascertain the extent of the lesion at the time the positive tissue diagnosis had been made. Finally, in a few instances we had to classify the staging by inference. These were patients who had not received treatment in our gynecology department.

TABLE VII. CLINICAL STAGING OF 175 CASES OF CARCINOMA OF THE CERVIX

Carcinoma in situ	81
Stage I	77
Stage II	10
Stage III	6
Stage IV	1
Total	175

One of the valuable results of the cancer survey program has been the opportunity to learn the types of management and therapy these 205 patients received during this preliminary study.

The outstanding achievement is the small amount of time which elapsed from the final positive tissue diagnosis until active therapy was started. In no instances (except for the 3 patients who had carcinoma in situ and were lost to follow-up by moving away from the county) could any cancer delay be attributed to physician or patient. Early therapy was started in all other cases.

In Table VIII will be seen the types and methods of treatment of the variously located carcinomas found in the 205 patients. The reader will disagree with some of these methods of therapy, and the authors of this presentation also believe strongly that some of the methods were unconventional and contraindicated.

A majority of the 205 patients who were found to have cancer are under the care of their own physicians, and treatment was administered by them or by surgeons in widely distributed hospitals in Columbus. There is evident a lack of standard and conventionally accepted therapy by the surgeons, radiologists, and gynecologists in Columbus and Franklin County who treat pelvic malignancy in the female.

Table VIII enumerates the therapy the 205 cancer patients received.

As originally stated, the cancer survey program of Columbus and Franklin County was initiated along similar lines to the Memphis program which began in June, 1952. One of the intentions of the sponsors, the United States Public Health Service, was to compare the data accumulated in other cities with those found in Memphis and Shelby County, Tennessee.

Although the fourth and latest Memphis report consisted of a 3½ year study, comprising over 140,000 women, the figures from their second report are used here for a closer comparative analysis between the two areas (Table IX).

In this preliminary report of the Columbus program, a difference in the incidence of cancer may be seen in comparison with the early study in Memphis. No definite conclusions can be drawn at this time, and many factors would have to be evaluated. Age of patients, parity, race, social status, climatic factors, and comparison of methods are variables that would have to be considered before any positive conclusions could be made regarding a more frequent occurrence of carcinoma in the female in Memphis than in Columbus.

On May 1, 1957, the one year repeat examinations were started on patients who had their first aspiration survey made one year ago. One year later, May 1, 1958, 11,190 recalls had been performed and the results are shown in Table

X. These 11,190 patients who returned for their second examination are from the total group of 35,394 women who were examined in the first year of the study. This represents approximately one woman out of 3 who returned for the second survey test.

The slides of all of these patients from their first survey one year earlier still were found on re-examination to be negative. Thus they did not fall in

TABLE VIII. TREATMENT IN 205 CASES OF CARCINOMA

Cervix—in Situ.—		
Total hysterectomy	54	
Amputation or cervicectomy	8	
Conization	6	
Biopsy—knife	6	
Cobalt^{60} plus total hysterectomy	1	
Cauterization	1	
Wertheim hysterectomy	1	
Observation (pregnant)	1	
None (lost)	3	
Total		81
Cervix—Stage I.—		
Cobalt^{60} and x-ray	17	
Radium	19	
Radium and x-ray	8	
Cobalt^{60} and Wertheim hysterectomy	13	
Wertheim hysterectomy	8	
X-ray	2	
Cervicectomy	2	
Total hysterectomy	7	
Trachelectomy and x-ray	1	
Total		77
Cervix—Stage II.—		
Cobalt^{60} and x-ray	8	
Radium and x-ray	2	
Total		10
Cervix—Stage III.—		
Cobalt^{60} and x-ray	2	
Radium and x-ray	3	
Total hysterectomy	1	
Total		6
Cervix—Stage IV.—		
No treatment		1
Vagina.—		
Wertheim hysterectomy and vaginectomy	1	
Radioactive gold seeds	1	
X-ray	1	
Total		3
Ovary.—		
Exploratory laparotomy, radioactive gold		1
Corpus.—		
Cobalt^{60} and total hysterectomy	12	
Radium and total hysterectomy	3	
Total hysterectomy	10	
Wertheim hysterectomy	1	
Total		26
Total		205

the false negative group. We believe this is a most significant finding and proves beyond doubt the value of periodic examinations at least once a year.

TABLE IX. COMPARISON OF MEMPHIS AND COLUMBUS STUDIES

	NO. EXAMINATIONS	CARCINOMA	
		NO. CASES	%
Memphis study	70,000	527 (1:143)	0.75
Columbus study	59,712	205 (1:290)	0.34

TABLE X. TYPES OF MALIGNANCY FOUND IN 11,190 RECALL EXAMINATIONS

Carcinoma in situ—cervix	6
Carcinoma—invasive—cervix	1
Carcinoma—endometrium	3
Total	10

Conclusions

Two years' experience in a survey program of cancer detection is short to draw conclusions that would be final and unequivocal. Certain definite impressions have been obtained, however, that substantiate the preliminary report:

1. A screening program is of distinct benefit in the earlier detection of cancer of the female genitals. In at least 73 per cent of the patients in whom cancer was found, the disease was not suspected. There is little doubt that the benefits of earlier diagnosis and the earlier treatment instituted should add to lengthened survival rates in these patients.

2. Earlier lesions of cancer are being found by cytological examinations. This becomes apparent when comparisons are made of earlier years' studies in the management of cancer in the female. An example of this progress may be seen by comparing the different percentages of clinical stages of cancer of the cervix 10 years ago with those detected by the cancer survey (Table XI).

TABLE XI. COMPARISON OF DISTRIBUTION OF CARCINOMA OF THE CERVIX AT THE TUMOR CLINIC OF THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY IN 1947 AND IN THE CANCER SURVEY 1957

CLINICAL STAGE	TUMOR CLINIC 1947 (%)	CANCER SURVEY 1957 (%)
0	0	38
I	14	48
II	41	5
III	41	7
IV	4	2

A community program such as that adopted and executed by vaginal aspiration or smear thus appears to discover earlier and even symptomless carcinoma of the uterus. These methods of earlier diagnosis and treatment offer increased hope for greater cure rates.

The incidence of carcinoma in situ of the cervix in the survey is most revealing. The number of patients with Stage 0 cervical carcinoma during the years from 1947 to 1956 in the Department of Obstetrics and Gynecology parallels the increase in the total number of cervical carcinoma patients registered. Its relation to the cancer survey project is shown graphically in Fig. 1.

The time of the institution of the project is shown by the arrow (1956). If percentages could be used to represent increase in the detection of this early lesion it would be a 325 per cent increase in the diagnosis of carcinoma in situ of the cervix. Such a fantastic increase, which is not statistically accurate, shows the tremendous possibilities of diagnosing early and curable cancer of the cervix by survey methods.

3. The aspiration and scraping techniques are suitable for large-scale screening. It should be emphasized that the test is not diagnostic. Its cardinal value is to point the way for further tissue-diagnosis procedure. It does not replace the biopsy, the conizing knife, or the curette. It supplements and aids in leading to these procedures.

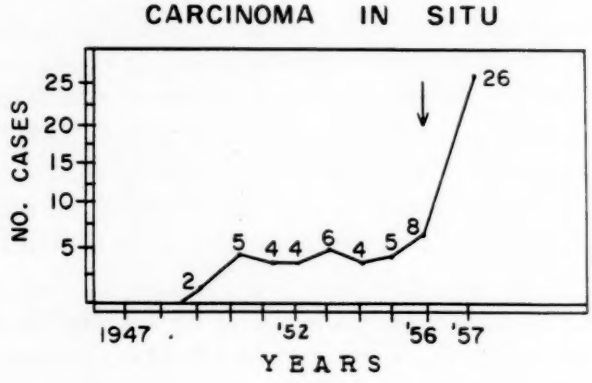

Fig. 1.

4. The aspiration technique has much value in both the clinically well and unsuspected patient, and the clinically suspicious patient. The scraping or smear technique is superior in diagnostic aspects.

5. Once the diagnosis has been made, early and more adequate therapy must be instituted. The study herewith presented sharply focuses the lack of uniformity in standard methods of therapy in pelvic malignancy. This is particularly true in carcinoma of the cervix. Physician education in cancer therapy must be advanced by continued programs for students in the medical colleges, and up through the interns, residents, general practitioners, and the specialists, including the general surgeons, radiologists, and gynecologists. Only by the combined methods of early diagnosis and proper therapy of cancer in the female can increasing success be achieved in the cure to which all of our patients are entitled.

We wish to express our deep appreciation to Drs. Emmerich von Haam and Edward Miller of the Department of Pathology, The Ohio State University, and Drs. Samuel C. Ingraham and Raymond F. Kaiser of the National Institutes of Health, Bethesda, Maryland, for their valuable contribution in the cancer survey project.

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Discussion

DR. CLYDE L. RANDALL, Buffalo, N. Y.—The critical question remains: How soon and how often may patients with negative findings and negative cytology be found to have developed carcinoma of the cervix during the interval between examinations? The Columbus figures include 7 new cases of cervical carcinoma among 11,190 women whose smears, taken by the aspiration technique, had been negative only one year before. This is an incidence of one apparently newly developed cervical cancer per 1,600 re-examinations.

In the office since 1948, employing only smears taken by the Ayre spatula in some 12,000 re-examinations after an interval of one or more years, we have to date found cervical carcinoma in only 3 women whose smears had previously been negative. Ring biopsies indicated that 2 were in the in situ state; one cervix was found with an early invading squamous-cell carcinoma slightly less than one year after the third of three annually negative smears. Thus it appears that not all cervical cancer can be trusted to mark time at the in situ stage for several years.

Dr. Ullery's report raises another question: What is the evidence that the detection of uterine cancer in favorably early stages actually promises improved results with presently available methods of treatment? Such evidence seems at hand in Gerhardt's[1] recent report concerning "Trends in Cancer Incidence in Mortality in the State of New York." Comparing the number of new cases of malignancy per 100,000 females in the state during the years 1942 and 1955, Gerhardt found only a 2 per cent increase in the number of new cases of cervical cancer being reported per year. During the same period of time there has been a 23 per cent increase in the number of new cases of corpus cancer, and a 15 per cent increase in the frequency of ovarian cancer among women in upstate New York.

While there seems to have been an actual increase in the frequency of gynecologic cancer developing per unit of population among women in the state, data very recently released by the Division of Cancer Control[2] indicate surprising trends in the number of deaths due to these same types of malignancy each year.

In the last 10 years, for instance, comparing the mortality rates for the years 1947 and 1956, deaths due to cancer of the breast decreased 2 per cent, deaths due to cancer of the cervix decreased 33.6 per cent, and deaths due to cancer of the corpus have decreased 27.5 per cent. Perhaps I should admit also that during this same 10 year period, deaths due to ovarian malignancy increased a startling 76 per cent and that it seems quite possible that too many ovaries are being preserved in upstate New York!

Today, however, we are interested in the 3.6 per cent decrease between 1947 and 1956 in the number of deaths due to carcinoma of the cervix in the state. It is my personal conviction that while earlier diagnosis obviously accounts for much of this improvement, we can also find evidence to suggest that, throughout this decade, one half to two thirds of all cases of cervical cancer detected in upstate New York received treatment in the Roswell Park Memorial Institute, the upstate hospital for the treatment of malignant disease. In all probability the uniformity and thoroughness of the treatment of a majority of these patients have contributed much to the accomplishment of this evidently improved result.

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DR. LEWIS C. SCHEFFEY, Philadelphia, Pa.—Dr. Ullery's paper continues and amplifies the initial report of a cancer survey project begun in 1956, organized and carried out with precision and able sponsorship. While in general it parallels the Memphis plan of population screening described successively by Erickson and his group since 1953, variations of definite value have been presented and further avenues of inquiry explored.

With respect to the women concerned, it might well be stated that the corollaries of mass screening tests are complete and periodic pelvic examinations, whatever the report of the cell test indicates. In other words, too much reliance may be placed by the "screened" individual on the statement that her test was "negative." This erroneous sense of security is brought out in Table X, as 10 pelvic malignancies were discovered among 11,190 patients or one third of the original group of 35,394 women returning for their recall test. Dare we speculate that a similar incidence might be found in the two thirds who did not respond to the recall? Also, does the "negative test" state of mind reduce concern for pelvic symptoms that might be present in an examinee harboring a known or unknown benign lesion? In this regard, Dr. Ullery has wisely and forcibly emphasized the need for qualification of the negative report given the patient in a survey reporting 97.7 per cent in this category.

One is impressed with the organization of a program that enlisted the splendid cooperation reflected in the work tables presented. Private physicians were responsible for 73 per cent of the slides received from 66,392 examinees (Table I). The importance of the doctor-patient relationship and the goal of making every physician's office a cancer detection center is further highlighted by the clinical superiority of the test when visualization and cervical scraping were used in addition to the single vaginal vault aspiration in approximately 13,150 patients, as delineated in Table V, revealing 18 patients with cancer suspected on smears negative with aspiration alone. The role of organized medicine in the Columbus project speaks well for its association with academic medicine and community relationships.

In the clinical review of the 205 pelvic malignancies (Table VI), indicating, I presume, the incidence of 0.34 per cent suspicious slides mentioned in Table II (one half those in Memphis), Dr. Ullery has specifically analyzed 175 cases of carcinoma of the cervix, Stages 0 to IV, among them. May I ask Dr. Ullery if he has an incidence figure for these? Only 3 patients, all of whom moved away, were lost to the follow-up and, although early therapy was started without delay in all the other patients, it was of a wide variety, especially with respect to the 81 women with carcinoma in situ (Table VIII). It would be of interest to know the ages of the 54 who underwent total hysterectomy and what was done with respect to the ovaries.

That earlier lesions are being found by cytology is revealed in Table XI as noted in the tumor clinic figures for 1947 when compared with the 1957 cancer survey figures. The indicative arrow in Fig. 1 shows progress indeed.

I would like to present some figures from the Uterine Cancer Cytology Research Project at the Woman's Medical College. Large groups of women employed industrially are being examined annually. To date 3,829 patients have been received and an incidence of 44 suspicious cervical tests (1.2 per cent) has been reported, compared with 1.8 per cent in Memphis. These investigators regard the incidence of carcinoma in a surveyed industrial population to be 5:1,000 on the basis of confirmed tissue studies. Unsuspected cancer confirmed by tissue study was 0.47 per cent (4.7 per 1,000), while in Memphis it was 0.5 per cent (5 per 1,000).

A. E. Rakoff and I in 1948 conducted a pilot study in patients attending the Donna Cancer Detection Clinics of Philadelphia. In 4,947 patients and among 13,329 cell tests, 7 invasive cancers were discovered, or 1.4 per 1,000, in a clientele predominantly of the Hebrew race. Correct positives were 85.7 per cent.

DR. CURTIS J. LUND, Rochester, N. Y.—There are three points that Dr. Ullery has made which I think need re-emphasis: (1) Cancer detection must be done in the office of the physician. We have screened at the present time approximately 75,000 patients in our Cytology Laboratory so our figures are rather comparable. We have not broken down our statistics as Dr. Ullery has in terms of private vs. clinic screening, but I feel certain the

figures will be approximately the same, as very few of our slides have come from the cancer detection clinics and most are from private physicians in the city. This is the way to diagnose cancer. (2) The incidence of histologically proved cancer in our experience has been about 1 per 500 smears rather than 1 per 300 as Dr. Ullery has reported. This is a small difference and it depends upon the histologic interpretation, and the population sampled, rather than the cytologic interpretation. (3) At the present time over half of all cases of cervical cancer admitted to our hospital is of the Stage 0 or intraepithelial type.

DR. HERBERT E. SCHMITZ, Chicago, Ill.—This study by Dr. Ullery and his associates brings out very clearly and strongly the advantage of screening groups of people who are under our control. Realizing that the perfect situation for such a screening project would be in the obstetric patient who comes in early in her pregnancy and visits her obstetrician regularly over a certain period of time, we instituted 3 years ago a routine screening of all patients admitted to the prenatal clinic. In this period we screened 4,244 patients and found 9 invasive cancers of the cervix and 5 carcinomas in situ. We assume that every cancer that shows up during pregnancy has been present since the first visit of the patient. Therefore, it is a most ideal situation for diagnosis and I recommend it to you.

DR. ULLERY (Closing).—I would say that the problem of security to the patient is indeed a real one. We are very concerned that the patients may think they are free of cancer or any possibility of it once they have had aspiration which is negative. The cards we send to them are very carefully worded. We tell them that the aspiration or the test they had done showed no evidence of malignancy but that that does not completely rule out the presence of malignancy; we tell them that if they have any unusual symptoms or signs by all means to come back to the clinic or see their doctor before the year is over. Even then it is difficult to brain wash the patient of the fact that she does not have cancer, but we try not to give a false sense of security.

The recall is another problem. Eleven thousand were recalled within a year's time. It was very difficult to do in Memphis and likewise in Columbus. We are giving the recall problem a great deal of publicity and hope to increase the number.

The incidence figures for cancer in the 205 patients that I showed you amount to about 30 per cent carcinoma in situ of the cervix and 40 per cent invasive of the cervix. If those are combined, it means that 70 per cent of the patients had either in situ or invasive cancer of the cervix.

Dr. Scheffey asked about the attitude toward hysterectomy or conservation of the ovaries in carcinoma in situ. We run into the problem that these patients are under the control of their own physicians, who refer them to other surgeons or do the operation themselves. The general attitude is in favor of total hysterectomy with a liberal vaginal cuff and conservation of the ovaries up to age 40. Over that age, total salpingo-oophorectomy is included with the hysterectomy.

Dr. Randall asked how soon and how often we should do follow-ups. This program was initiated by the National Institutes of Health to do one aspiration a year. We do not feel that is enough. We certainly believe that every patient should at least have a periodic examination every 6 months and oftener if untoward symptoms arise.

It is too early to give any conclusions about the results of therapy, and I did not give the mortality rate in the 205 patients because 2 years is too short a time. We have had 3 patients who have died: One had a Stage IV cancer and was in extremis at the time she was seen. A second patient had a total hysterectomy alone for a Stage III carcinoma and she died within a week, and the third patient had a total hysterectomy performed for carcinoma in situ and developed pulmonary embolus after the operation.

I agree heartily with Dr. Schmitz's comments on the value of screening obstetric patients. In our clinic at the University Hosptial we perform a vaginal aspiration every trimester during pregnancy with the initial aspiration and smear on the first visit. We have found 2 invasive cancers of the cervix and 1 in situ. We think the obstetric patient is ideal for screening study.

THE FOLLOW-UP OF PATIENTS WITH CANCER OF THE CERVIX TREATED BY RADICAL HYSTERECTOMY AND RADICAL PELVIC LYMPHADENECTOMY*

BAYARD CARTER, M.D., ROY T. PARKER, M.D., WALTER L. THOMAS, M.D., ROBERT N. CREADICK, M.D., CHARLES PEETE, M.D., WALTER B. CHERNY, M.D., AND JEAN B. WILLIAMS, M.D., DURHAM, N. C.

(From the Department of Obstetrics and Gynecology, Duke University School of Medicine and Duke Hospital)

THIS report is an addition to the two previous reports made by our group[1, 2] in 1948 and 1952. The reports in 1948 and 1952 gave the plan of study of radical hysterectomy and radical pelvic lymphadenectomy in the treatment of invasive squamous-cell cancer of the cervix or of the cervical stump. Included in these reports was our experience with this operation in the treatment of adenocarcinoma of the cervix and of the cervical stump. This was done deliberately to give the opportunity to compare the salvage rates in the two cancers. This method is also followed in the present report.

No attempt is made to review the voluminous literature.

Purpose of This Report

The main purpose of this report is to give the *over-all salvage* we have noted as the experiment continues. The present report covers the patients treated from 1944 through 1952 and thus affords follow-ups for from 5 plus years to 13 plus years. The deaths include deaths from all causes.

We continue to divide the patients into four main groups:

1. Patients who received no preoperative irradiation therapy.

2. Patients who received preoperative x-ray therapy alone or radium therapy alone.

3. Patients who received preoperative "complete" and "adequate" x-ray and radium therapy, as we understand the therapy.

4. Patients who received preoperative x-ray and radium therapy which we could not accept as "complete" and "adequate" therapy.

In determining the over-all salvage from 5 plus to 13 plus years, we have not attempted to determine the effect which irradiation, given preoperatively or postoperatively, had on the course of the disease, on the complications of treatment, or on the over-all salvage. This will be the subject of a subsequent report.

*Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

The first two reports, and this one, do not include the radical hysterectomies and radical pelvic lymphadenectomies done for adenocarcinoma of the endometrium or for choriocarcinoma. They do not include the exenteration operations done for invasive squamous or adenocarcinoma of the cervix or of the cervical stump, for adenocarcinoma of the endometrium, for primary or secondary squamous-cell or for secondary adenocarcinoma of the vagina, for cancer of the bladder or of the bowel with pelvic involvement, for "persistent" or "recurrent" cancers in any site in the pelvis, for cancer of the ovary, or for cancer of the vulva.

Table I shows the squamous-cell cancers and the adenocarcinomas of the cervix or of the cervical stump from 1944 through 1957, and lists the operations for each year.

TABLE I. THE YEARLY INCIDENCE OF CANCER OF THE CERVIX AND OF THE CERVICAL STUMP AND THE NUMBER OF OPERATIONS

YEAR	TOTAL CASES CANCER CERVIX	TOTAL OPERATIONS	% OPERATIONS
1944	145	2	1.37
1945	157	16	10.19
1946	146	27	18.49
1947	140	38	27.14
1948	165	31	18.78
1949	173	33	19.07
1950	142	21	14.78
1951	115	20	17.39
1952	128	9	7.00
1953	85	19	22.00
1954	91	16	17.5
1955	113	11	9.07
1956	107	22	20.55
1957	95	12	12.62
Total	1802	277	15.4

This salvage report covers the first 177 operations for squamous-cell cancers and 20 operations for adenocarcinomas of the cervix or of the cervical stump performed from 1944 through 1952. This makes possible a statement of the over-all salvage for a period of 5 plus years to 13 plus years.

We had hoped to show a relatively high salvage for patients with Stage I and Stage II cancers for a longer period than 5 years. We also hoped that the salvage of patients with positive nodes might be relatively high.

Summary of Previous Report

In the 1952 report the uncorrected 5 plus year salvage in 27 patients with Stage I squamous cancer of the cervix was 26 (96.3 per cent). Two of these 27 patients had positive nodes and lived 5 plus years. In the 15 Stage II cases of squamous-cell cancer the 5 plus year salvage was 11 (73.3 per cent). Four of the 15 Stage II patients had positive nodes and 3 (75 per cent) lived 5 plus years. The total uncorrected 5 plus year salvage for Stage I and Stage II was 37 (88.0 per cent).

Four patients with Stage I adenocarcinoma showed an uncorrected 5 plus year salvage rate of 3 (75 per cent). One of these patients had positive nodes. In 4 patients with Stage II adenocarcinoma the 5 plus year salvage was 0. Two of these 4 patients had positive nodes. The Stage IV adenocarcinoma patient had positive nodes and died. The total uncorrected 5 year plus salvage rate for the 9 adenocarcinoma patients was 3 (33.33 per cent). Of the 9 patients 4 (44.4 per cent) had positive nodes. The salvage in the 4 patients with positive nodes was one (25 per cent) and that was a Stage I.

Table II shows the incidence of cancer of the cervix and the incidence of node metastases in patients who received no preoperative irradiation and in patients who did receive preoperative irradiation. The total number of patients was 277 in the years 1944 through 1957.

TABLE II. INCIDENCE OF CANCER OF CERVIX AND REGIONAL NODE METASTASES WITH AND WITHOUT PREOPERATIVE X-RAY AND RADIUM

PREOPERATIVE TREATMENT	PATIENTS		ACTIVE CANCER CERVIX		ACTIVE CANCER NODES	
	NO.	%	NO.	%	NO.	%
No irradiation	121	43	115*	95	23	19
Adequate x-ray and radium	53	19	13	25	8	15
Adequate x-ray	67	24	33	49	15	22
Adequate radium	25	9	6	25	4	16
Inadequate irradiation	11	4	5	45	3	27
Total	277		172		53	19.1

*Six patients with invasive carcinomas in cold knife conization specimens and negative cervices in radical operation specmens.

Table III shows the incidence of node metastases in the clinical stages of patients who did, and patients who did not, receive preoperative irradiation. Of the 277 patients during the years 1944 through 1957, 53 (19.1 per cent) had positive nodes.

TABLE III. INCIDENCE OF NODE METASTASES IN THE CLINICAL STAGES WITH AND WITHOUT IRRADIATION THERAPY

STAGE	TOTAL PATIENTS		REGIONAL NODE METASTASES					
			NO IRRADIATION			WITH IRRADIATION		
			OPERATIONS	CANCER IN NODES		OPERATIONS	CANCER IN NODES	
	NO.	%	NO.	NO.	%	NO.	NO.	%
I	178	64	104	15	14	74	6	8
II	88	31	16	7	43	72	20	27
III	9	3	1	1	100	8	2	25
IV	2	0.7	0	0	00	2	2	100
Total	277		121	23	19	156	30	19

TABLE IV. SUMMARY OF SURVIVAL IN 177 SQUAMOUS-CELL CANCERS AND 20 ADENOCARCINOMAS FOR THE YEARS 1944 THROUGH 1952

YEAR	SURVIVAL	TOTAL PATIENTS	LIVING		DEAD OF CANCER		DEAD OF OTHER CAUSES	
			NO.	%	NO.	%	NO.	%
1944	13 plus years	2	2	100	0		0	
1945	12 plus years	16	14	87.5	0		2	
1946	11 plus years	27	22	81.4	3		2	
1947	10 plus years	38	24	63.1	11		3	
1948	9 plus years	31	19	61.2	10		2	
1949	8 plus years	33	21	63.6	6		6	
1950	7 plus years	21	18	85.7	2		1	
1951	6 plus years	20	13	65.0	6		1	
1952	5 plus years	9	7	77.7	1		1	
Total		197	140	71.0	39	19.8	18	9.1

Salvage by Years, 1944 Through 1952

As stated in the introduction, the purpose of this report is to give the over-all salvage in the 197 patients who had operations from 1944 through 1952. Of the 197 patients there were 39 (19.8 per cent) patients with positive nodes (Table IV).

A summary of each year from 1944 through 1952 is also given in Table IV, showing the salvage for each year, as well as the deaths from cancer and deaths from other causes.

1944.—Two patients were operated upon. The nodes were negative. Both patients have lived 13 plus years.

1945.—Sixteen patients were operated upon; 14 are living 12 plus years later. Twelve had negative nodes and 2 had positive nodes.

Two patients died of other causes. One, with Stage I squamous cancer and with negative nodes, died elsewhere 3 years later of intestinal obstruction for which diagnosis and treatment were delayed. One, with Stage II squamous cancer and with negative nodes died at 12 plus years of subacute bacterial endocarditis. There was no demonstrable cancer at the time of death.

1946.—Twenty-seven patients were operated upon and 22 are living 11 plus years. Of the 22 living patients there were 2 patients with positive nodes.

Three patients died of cancer. One with Stage I squamous cancer with positive nodes died in 18 plus months. One with Stage II squamous cancer with negative nodes died in 15 plus months. One with Stage II adenocarcinoma with positive nodes died in 10 plus months.

Two patients died of other causes. One with Stage II squamous cancer with negative nodes died in 16 plus months of bowel complications attributed to operation. One patient with Stage I squamous cancer with negative nodes died elsewhere at 5½ years of intestinal obstruction for which diagnosis and treatment were delayed.

1947.—Thirty-eight patients were operated upon and 24 are living 10 plus years.

Eleven patients died of cancer, 7 of squamous-cell cancer and 4 of adenocarcinoma. One with Stage I squamous cancer and negative nodes died in 10 plus months. Although the nodes were negative for cancer, the diagnosis of Boeck's sarcoid was made. One with Stage I squamous cancer and negative nodes died in 18 plus months of metastatic lung cancer. One patient with Stage II squamous cancer with positive nodes died in 6 plus months. Another with Stage II squamous cancer with positive nodes died in 24 plus months, and a third in 18 plus months. One patient with Stage III squamous cancer with positive nodes died at 6 plus years of primary cancer of the bronchus and one with Stage I squamous cancer with positive nodes died in 6 plus years of metastatic cancer.

Four patients with adenocarcinoma died, one with a Stage II adenocarcinoma with negative nodes in 33 plus months; one with Stage II adenocarcinoma with positive nodes in 1 plus years; and one with stage IV adenocarcinoma with positive nodes in 1 plus years. The latter patient had received preoperative irradiation elsewhere. One patient with Stage I adenocarcinoma with negative nodes died in 5 plus years of the cancer.

Three patients died of other causes. One patient with Stage II squamous-cell cancer with negative nodes died in 9 plus years of pyelonephritis which was attributed to her treatment; another with Stage II squamous cancer with negative nodes died in 6 plus months of uremia which was attributed to her treatment; and one with Stage II adenocarcinoma with negative nodes died in 3½ years of severe diabetes and hypertensive cardiovascular renal disease. There was no evidence of cancer at the time of death.

1948.—Thirty-one patients were operated upon and 19 are still living 9 plus years later.

Ten patients died of squamous-cell cancer: 3 with Stage I squamous cancer with negative nodes lived 3 plus, 4½ plus, and 5 plus years; 3 with Stage II squamous cancer with positive nodes lived 9 plus months, 10 plus months, and 3 plus years; one with Stage II squamous cancer with negative nodes lived 4 plus years. One patient with Stage III squamous cancer with negative nodes died in 18 plus months. She had received preoperative irradiation therapy. Another with Stage III squamous cancer but with positive nodes died in 9 plus months. She had received preoperative irradiation therapy. One with Stage IV squamous cancer with positive nodes died in 9 plus months. She also had received preoperative irradiation therapy.

Two patients with Stage I squamous cancer and negative nodes died of other causes: One lived 5 plus years with no evidence of cancer and the cause of her death, which was sudden, was never clearly defined by her physician. One lived 3 plus years with no evidence of cancer but died of pyelonephritis and uremia which were attributed to her treatment.

1949.—Thirty-three patients were operated upon and 21 are living 8 plus years.

Six patients died of cancer, 5 of squamous-cell cancer and one of adenocarcinoma. One patient with Stage II squamous-cell cancer with negative nodes died in 22 plus months; 4 with Stage II squamous-cell cancer with positive nodes died in 1 plus years, 1 plus years, 2½ plus years, and in 5 plus years; and one patient with Stage I adenocarcinoma and positive nodes died in 7 plus months.

Six patients with squamous-cell cancer died of other causes: One with Stage I cancer with negative nodes died in 19 days of a transfusion reaction and another with Stage I cancer with negative nodes died in 4 plus months of pelvic cellulitis. These 2 are the only immediate operative deaths. Two patients with Stage I cancer with negative nodes died, one in 3½ plus years of pyelonephritis and hypertensive disease which were attributed to her treatment, and the other in 6 plus years of pulmonary tuberculosis. There was no evidence of cancer at the time of her death. One with Stage II cancer and negative nodes died in 2 plus years of pulmonary tuberculosis. There was no evidence of cancer at the time of her death. Another with Stage II cancer and negative nodes died in 5½ plus years of pyelonephritis which was attributed to her treatment.

1950.—Twenty-one patients were operated upon and 18 are still living 7 plus years later.

Two patients with squamous-cell cancer died of cancer: One with Stage II cancer and positive nodes died in 16 plus months. The other with Stage II cancer but with negative nodes died in 18 plus months.

One patient died of other causes. She had Stage I cancer with negative nodes and died in 12 plus months of kidney disease due to bilateral ureteral obstruction which was attributed to her therapy.

1951.—Twenty patients were operated upon and 13 are living 6 plus years.

Six patients died of squamous-cell cancer: One with Stage I disease with negative nodes died in 19 plus months. One with Stage I cancer, who was 3 plus months pregnant at the time of operation and who had positive nodes, died in 3½ plus years. Four with Stage II cancer with positive nodes died in 12 plus months, 12 plus months, 16 plus months, and in 5 plus years.

One patient with Stage II squamous-cell cancer and negative nodes died in 10 plus months of ureteral obstruction and kidney disease which were attributed to her treatment.

1952.—Nine patients were operated upon and 7 are living 5 plus years. One who had Stage I adenocarcinoma with positive nodes died in 7 plus months of cancer.

One patient died of other causes. She had Stage I squamous cancer with negative nodes and died in 5½ plus years of bronchogenic cancer. This was considered a primary cancer in that it was found in a metaplastic area in the bronchus. She showed no evidence of pelvic cancer.

TABLE V. SALVAGE BY STAGES IN 177 CASES OF SQUAMOUS-CELL AND 20 CASES OF ADENOCARCINOMA OPERATED UPON FROM 1944 THROUGH 1952 WITH NUMBER OF DEATHS FROM EACH

	OPERATIONS		LIVING		DEAD			
					SQUAMOUS		ADENOCARCINOMA	
STAGE	NO.	%	NO.	%	NO.	%	NO.	%
Stage I	119	60.4	97	81.5	19	15.9	3	2.5
Stage II	68	34.5	39	57.3	25	36.7	4	5.8
Stage III	8	4.0	4	50.0	4	50.0	0	0.0
Stage IV	2	1.0	0	00.0	1	50.0	1	50.0
Total	197	100.0	140	71.0	49	24.9	8	4.0

187 Total Stage I and Stage II
Total Living Stage I and Stage II
136 72.7 per cent

Summary

In Table V the 197 cancers are divided into the various stages and the salvage for each stage is shown. The total number of deaths from squamous-cell cancer and from adenocarcinoma is also shown.

For squamous-cell cancer 177 operations were done; 33 (18.6 per cent) patients had positive nodes. The uncorrected 5 plus to 13 plus year salvage in these 33 patients was 12 (36.3 per cent). Deaths from all causes in the 177 patients numbered 49 (28.6 per cent). The uncorrected salvage was 128 (72.3 per cent).

For adenocarcinomas 20 operations were done; 6 (30 per cent) patients had positive nodes. One patient (16.6 per cent) with positive nodes has lived 13 plus years. There were 8 (40 per cent) deaths from all causes in the 20 patients. The uncorrected salvage in the 20 patients operated upon was 12 (60 per cent).

If the 33 (18.6 per cent) patients with positive nodes were excluded from the 177 cases of squamous-cell cancer, in the remaining 144 patients there were 28 (19.4 per cent) deaths in the period of 5 plus to 13 plus years. This would give an uncorrected salvage in all patients with negative nodes of 116 (80.6 per cent).

If the 6 (30 per cent) patients with positive nodes were excluded from the 20 patients with adenocarcinoma there would be 2 deaths (14 per cent). This would give an uncorrected salvage in all patients with negative nodes of 12 (86 per cent).

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Discussion

DR. CONRAD G. COLLINS, New Orleans, La.—These results in patients with squamous-cell cancer and negative nodes are as good as or better than results achieved in other

clinics where radiation therapy alone is employed. The authors' results in patients with squamous-cell cancer and positive nodes, namely, a 36 per cent salvage, are excellent for this group. In adenocarcinoma of the cervix, metastasis is more frequent and the results not as good as when dealing with squamous-cell carcinoma. Operative mortality was negligible in their cases.

Prior to 1952, the year of their last report, we find there were 881 cases of cancer of the cervix encountered and 170 operations (19 per cent) performed. During the years 1952 to 1957, inclusive, there were 619 cases of cervical cancer surveyed and 91 operations (15 per cent). Is this decrease in the utilization of operation due to an increase in the number of Stage III and Stage IV cancers encountered, or is it due to a reappraisal concerning the limits of surgery as gleaned from their previous survey? Furthermore, the salvage rate in the years 1944, 1945, and 1946 was 89 per cent; in the years 1947, 1948, and 1949 it was 63 per cent, and in 1950, 1951, and 1952 it was 76 per cent. Is this decrease in salvage (1947-1952 inclusive, 69 per cent) due to an increase in the number of cases operated upon without prior irradiation therapy? Or were more cases of Stage III and Stage IV cancer operated upon and procedures less than anterior, posterior, or total exenteration employed for the disease at these stages?

Of significance is the difference noted in the number of positive nodes in patients who had irradiation prior to operation and those who had not. It appears that preoperative irradiation does have a beneficial effect not only as regards the local lesion but also nodal metastasis.

On the Tulane Unit, Charity Hospital, we still regard irradiation as the treatment of choice in the therapy of invasive cancer of the cervix and, with the exception of cancer of the cervix complicated by pregnancy, utilize radium and x-ray in every case. We believe that the therapy of recurrences is surgery, whether this be an extensive hysterectomy and pelvic node dissection or anterior, posterior, or total exenteration. We urge the patients with recurrences and with the disease process limited to Stage I or II to have the former. In Stages III and IV, the extent of the procedure of exenteration and our mortality and survival rates are explained to the patient and she either accepts or rejects the procedure. We have not arrived at the point where we urge the patient to accept total or partial exenteration. In addition, as a study in Stages I and II, we have performed extensive hysterectomy and pelvic node dissection electively in patients who have had irradiation therapy. Not all patients accept operation and some are discouraged from doing so by our confreres in the department of radiotherapy.

Thus our series of cases of extensive surgery for cancer of the uterus does not in any way include the large numbers reported by the Duke team. Since January, 1948, we have performed 150 operations. Fifty of these were exenterations. Seventy-nine extensive hysterectomies and pelvic node dissections were performed for invasive carcinoma of the cervix. The remainder were for other malignancies of the uterus. Of the 79 patients, all were in Stage I or II with the exception of 6 cases classified as Stage III. In Stage I and II, 10 patients (100 per cent) are alive less than one year following operation; 21 are alive and 1 is dead (95 per cent) one to 3 years following operation; 16 (80 per cent) are alive and 4 dead 3 to 5 years following operation; and 14 patients (82 per cent) are alive and 3 dead 5 or more years following operation. The incidence of node metastasis was 11 per cent.

Recurrence was determined by biopsy of the cervix. Lately we have been using the Silverman needle for biopsy of the parametrium. Cortisone is also being employed in an attempt to distinguish between parametritis and infiltration with carcinoma. It is too early for us to evaluate these methods but they seem promising. We do not have the facilities for the study of SR or RR.

Our incidence of ureteral fistulas is 10 per cent. This is mentioned only because it is slightly higher than that encountered in reports where preoperative irradiation had not been used. As to the difficulty of operation, we cannot express any opinion as to whether it is more difficult to operate upon a patient who has previously been irradiated or not for

we irradiate and then operate. The time interval between the two procedures varies from 4 months to 2 years. Suffice it to state that the majority of these operations were performed by our residents, under supervision, and if they can do the difficult the easy will be no problem. These cases were not selected with regard to age, medical complications, etc.

We are sorry that Dr. Carter has not included his fistula rate and think that a comparison of the rate in patients who have had previous irradiation and in those who have not is very important.

DR. LANGDON PARSONS, Boston, Mass. (read by Dr. Joe V. Meigs)—This excellent 10 year salvage points out something that I believe is very important about the life history of cancer of the cervix. In discussing end results one is accustomed to talk in terms of 5 year results. I am convinced that a 3 year salvage figure is significant in evaluating cancer of the cervix in contrast to cancer of the breast, where I doubt that a five year figure means very much. Only 7 patients in the 197 cases reported upon here died of recurrent disease 3 years after the operation. The great majority died with disease within 3 years or later when death was due to complications or intercurrent disease.

You will note in Dr. Carter's statistics that he is seeing one third fewer cases than he did 10 years ago, although he operates upon the same percentage. His experience parallels ours. Perhaps the incidence of cancer of the cervix has declined, but I suspect that many operations are being done in the smaller community hospitals where the surgery may or may not be adequate. To be adequate the paracervical and paravaginal lymphatics must be removed. To my mind this is more important in salvage than the node dissection.

I do not wish to imply that the node dissection is not essential. I believe you can cure over 50 per cent of patients in Stage I who have positive nodes. I am less convinced that you can do it in Stage II. Dr. Carter's figures show an over-all salvage of 36 per cent in both Stages I and II where nodes are involved. He does not mention the number of positive nodes in each stage but it is interesting to note that the great majority of deaths in the Stage II group came in patients with positive nodes.

Not only must you do a proper operation but you must do it on the right patient. Today too many inadequate operations are being done on patients who have too much disease and might better have been treated by radiation. The Wertheim operation with pelvic node dissection is designed adequately to encompass disease in Stage I and Stage IIa but I question very much whether it is adequate for IIb.

The study of large block sections of Wertheim and exenteration specimens is very revealing. In many instances there is more remote occult disease than you can determine with your examining finger. I tried, therefore, a surgical experiment in all stages of disease in 100 cases, modifying the surgical procedure to the amount of disease believed to be present. Of these cases, 86 were operated upon over 5 years ago. When the Wertheim was done for Stages I and IIa, the salvage rate was 84 per cent; including the cases in Stage IIb brought the salvage to 93 per cent, and in Stage II alone, there was a 54 per cent 5 year cure rate.

In 11 cases, I tried to extend the Wertheim to Stage IIb but salvaged only 3 patients. It is not a question of being able to perform the operation, but the recurrences and the morbidity came in this group. Recognizing this, I then performed anterior exenterations in late Stage IIb and Stage III, salvaging 2 of 9 patients. In Stage IV, 2 of 7 patients were salvaged by total exenteration.

I am not advocating exenteration procedures as primary definitive therapy for cancer of the cervix, but if we are to do surgery we must have another string to our bow to care for the patient who proves to have more disease than we originally believed.

It does, I think, point out that if your operation is adequate to encompass all the disease, you have a chance to win but you cannot make it do more than it is supposed to do.

DR. JOE V. MEIGS, Boston, Mass.—Dr. Carter has shown there is a place for surgery in the treatment of cancer of the cervix. I was one of the early ones to recommend this form of treatment and I believe that it is a very important method of treatment. I believe also in radiation therapy but it cannot be stated that the only treatment is radiation. I believe

you will lose many patients if you avoid surgery. Dr. Miller has shown in his work and Ruth Graham has also shown that some of the patients are resistant to radiation. Our job is to find which patient is which and to do the correct thing for the patient.

Table I shows the 10 year results in cancer of the cervix and I believe they show that surgical treatment stands up well. Of the 97 patients, 69 per cent with Stage I cases are living and 58.1 per cent with Stage II. Table II gives information on results in cases with positive nodes. Of the 97 patients, 22.7 per cent are living at the end of 10 years; of Stage I, 27 per cent with positive nodes are alive for ten years. Perhaps radiation can do the same thing. I believe radiation will destroy lymph nodes in the proper patient. Table III is an attempt to show what surgery alone can do. I have had many discussions with our radiation therapist, and he says you have to include in the total number you operate upon the patients in whom surgery failed and who had radiation after operation. In our series, 44 had operation only and 84 per cent are well at 10 years but there were 7 who were failures who had radiation therapy. We must add those to the operations but we cannot add the cured patients. Therefore, 72 per cent with straight surgery are alive at 10 years. This is a demonstration of what straight surgery can do.

Fistulas developed in 9.3 per cent of the patients. Of these 6 healed spontaneously. Five healed with loss of a kidney and one healed in continuity. I used to worry about fistulas but I don't so much any more, because if you do a good job and still get a fistula it is unfortunate, but if you try to save the ureter by leaving tissue around it, that is poor surgery. I would rather have a patient living with a fistula than with persistent disease as a result of leaving tissue which should have been removed.

Six patients died of intercurrent disease; 5 other patients died 5 years and 3 of these of cervical cancer.

I am keenly interested in the surgical treatment but I believe radiation treatment also is important.

TABLE I. TEN-YEAR RESULTS

	NO. CASES	LIVING	PER CENT
Stage I	66	47	71.2
Stage II	31	18	58.1
Total	97	65	67

TABLE II. TEN-YEAR RESULTS

	NO. CASES	POSITIVE NODES		LIVING	
		NO.	%	NO.	%
Stage I	66	11	17.0	3	27.0
Stage II	31	11	35.0	2	18.0
Total	97	22	22.7	5	22.7

TABLE III. TEN-YEAR RESULTS

	NO. CASES	LIVING		POSITIVE NODES		
					LIVING	
		NO.	%	NO. CASES	NO.	%
Operation only	44	37	84	7	3	42
Surgical failure	7	1		4	0	
Total	51	37	72	11	3	27

DR. SUBODH MITRA, Calcutta, India.—I would like to present the end results of the operation designed be me for cancer of the cervix, namely, the extraperitoneal pelvic lymphadenectomy and radical vaginal hysterectomy.

During the last 7 years, I operated upon 192 cases, of which 98 cases belonged to Stage I, 72 to Stage II, and 22 to Stage III.

It is often said that radical vaginal hysterectomy cannot be done if there are associated pelvic lesions. Out of 420 radical vaginal hysterectomies performed by me, associated pelvic lesions such as tuboovarian mass, endometriosis, pyosalpinx, and so forth, were encountered and not a single case was found inoperable by the vaginal route.

In 25 per cent of the total cases, positive pelvic nodes were found, including 18 per cent in Stage I.

Curiously enough, the parametrium was found infiltrated with cancer cells in only 14 per cent of cases as compared with 25 per cent nodal involvement.

The 5 year salvage was 61 per cent for all stages and 64.5 per cent for Stages I and II.

Breaking down the results of node-positive and node-negative cases, the 5 year cure rate was 66 per cent in gland-negative cases and 44 per cent in gland-positive cases.

There is a striking difference in end results between this new operation and Schauta's operation previously done by me, namely, a 61 per cent 5 year cure rate with the new operation as against 44.5 per cent using Schauta's technique.

The corrected primary mortality is 1 per cent. There was no ureteral fistula and residual urine was reduced to 3 oz. within 10 days.

DR. HAYAMI FUJIMORI, Osaka City University, Japan.—I have developed a new method for the diagnosis of cancer of the cervix with radioactive phosphorus. This new technique consists of the administration of P^{32} in the form of sodium acid phosphate 1 to 5 μc per kilogram of body weight in 20 c.c. of 20 per cent glucose. This is given in the femoral artery while it is simultaneously occluded distally, thus forcing the tracer solution retrograde into the uterine arterial circulation without passing through the rest of the body, and bringing a much higher concentration of P^{32} to the lesion with a lower total dosage.

Radioactive phosphorus was administered in this manner to 19 patients with proved cervical cancer (including 6 cases of carcinoma in situ) and 11 with benign erosion or no lesion of the cervix. At 2, 4, 12, and 24 hours after the injection, a biopsy was taken, dry ashed, and the radioactivity measured with a Geiger-Muller counter. Simultaneously blood samples were drawn and the radioactivity of the dry ashed serum was measured. Expressed in counts per minute per germ of tissue or serum, the radioactivity of the cervical biopsy was tabulated as percentage of the radioactivity of the serum. We found the 4 hour specimen to be the most significant. In 14 of 19 cases (73 per cent) with proved malignancy, there was a value of more than 12 per cent of the serum value, while in the 9 cases without malignancy, 2 (22 per cent) gave a value of over this figure. It is of great interest that 4 of the 6 cases of carcinoma in situ gave a value higher than the demarcation level of 120 per cent. The percentages of false positive and false negative in this test compare favorably with the results of Papanicolaou's test.

DR. MILTON L. MC CALL, New Orleans, La.—I am sorry Dr. Carter did not mention something about the complications and, more specifically, ureterovaginal fistula. It has been well established that modern-day radical surgery for cancer of the cervix is associated with low mortality. The development of such serious morbidity as ureterovaginal fistula is not uncommon, however. In fact, most of the better clinics doing radical surgery report an incidence of approximately 8 to 20 per cent for this complication. In my last 63 radical abdominal hysterectomies with pelvic gland dissection, there were 6 fistulas; 3 were ureterovaginal, and 3 were vesicovaginal. About one-half of these patients had been irradiated before operation, and the other half had not. I am not sure what part irradiation plays in the development of fistulas. Collins of Tulane feels it is not important although most other authors on the subject feel it is a significant factor.

During the past 23 months, this discussant has performed 60 Schauta-Amreich radical vaginal operations, utilizing the technique of Professor Ernst Navratil of Graz, Austria. A detailed preliminary report of these cases will be made within the near future. One of the reasons for reinvestigating this interesting technique, which has never been popular in the United States, was to study the incidence of ureterovaginal fistula, as well as other types

of serious morbidity, as compared with such complications following the radical abdominal operation or complete irradiation. All patients except those with early stromal invasion were also subjected to bilateral extraperitoneal lymphadenectomies.

There have been no fistulas of any kind in 60 patients who have been operated upon with the radical vaginal technique. The ureter is dissected out for a distance of 8 to 10 cm. in most cases. The approach to the ureter is quite different from that at the abdominal operation. The distal 6 or 8 cm. is completely dissected out on all sides. Above this, the ureter is released from all surrounding tissues laterally as well as inferiorly as the patient lies in lithotomy position. Viewing the ureter from below, we see that it remains attached superiorly to the peritoneum of the pelvis above this point. In the abdominal operation, the ureter is attacked from exactly the opposite direction, and there is often significant bleeding, and, I feel, much more trauma no matter how carefully the dissection is carried out. The vaginal approach to the ureter may ultimately be shown to be the safest after all, in which case either the Schauta technique or a combined vaginal-abdominal procedure would become a necessary part of the operative armamenterium for all those who treat cancer of the cervix surgically.

DR. CARTER (Closing).—This was a report on uncorrected salvage and had no analysis of the complications. We hope to make the complications the subject of a subsequent report. We do get fistulas. Our uncorrected fistula rates over the period of 5 plus to 13 plus years were: (1) uterovaginal fistulas, 8.9 per cent; (2) vesicovaginal fistulas, 7.2 per cent; (3) combined fistulas in 2 patients. Fistulas due to invasive cancer totaled 2.1 per cent. One ureteral fistula was caused by ureteral catheterization during an attempt to dilate a ureteral stricture.

We had high hope in our first report of salvage for 5 years in Stage I and Stage II cancers. We feel strongly that in time we will come to some method of treatment, either by irradiation or by operation or by a combination of the two, which will permit removal or destruction of all cancer-bearing tissue. We wish we could feel that irradiation destroys the cancer in the nodes. We feel skeptical about any attempt on our part to predict which patient is going to be "cured" by irradiation therapy.

In answer to Dr. Mitras' question concerning positive nodes we would state that we were very hopeful following our first report. However, the figures given in the summary represent a salvage for 5 plus to 13 plus years and show that for squamous-cell cancer the uncorrected salvage for patients with positive nodes was approximately 36 per cent.

We have tried every method to detect "persistent" cancer in the cervix and in the nodes but the only definite answer we have found is to remove tissue for examination.

Our report admittedly covers a selected series of patients. The only factors which contraindicated the operation were gross obesity and the wish of the patients. Once the nature of the operation has been explained, many patients elect to have irradiation therapy.

URETHRAL OBSTRUCTION IN THE FEMALE CHILD*

C. Bernard Brack, M.D., and
Harriet G. Guild, M.D., Baltimore, Md.

(From the Departments of Gynecology and Pediatrics, Johns Hopkins University and Hospital)

THE anatomy, pathology, and symptomatology of the female urethra have been the subject of widely divergent opinions among competent urologists. Some authorities have greatly emphasized the importance of obstructive lesions of the urethra and vesical neck, while others have minimized the importance of these regions in the production of symptoms.

That persistent urinary tract infection in female children is potentially very serious has been pointed out by a number of writers, including one of us. After a follow-up study of 30 cases of pyelitis in infancy and childhood, Wharton, Gray, and Guild[10] found that in only 13 cases was the urinary tract entirely normal. Their observations were later confirmed by Woodruff and Everett.[11]

Congenital urethral valves are said to be very rare in the female. In 1936 William E. Stevens[8] was able to find in the literature since 1552 only 14 cases of obstruction of the female urethra by diaphragms or valves. Complete urethral obstruction was present in 5 stillborn infants, and another infant with complete obstruction died 8 days after birth. Four infants were cured by perforation of the obstruction.

Partial obstruction of the urethra in children by lesions other than valves appears to be somewhat more common. Campbell reports that 10 suprapubic bladder neck resections and 28 transurethral procedures were used in the treatment of 512 cases of hydronephrosis. It is not stated, however, how many of these little patients were females. The same author refers to "polyps" as one variety of acquired lesion causing urethral obstruction in children.

For many years, members of the Department of Gynecology at Johns Hopkins have condemned the use of the resectoscope in the female. This attitude resulted from the observation that many cases of urinary incontinence followed transurethral resection. In 1948 Everett[4] wrote, "Benign obstructive lesions at the vesical neck sufficient to require or justify the use of the cautery punch or resectoscope rarely if ever occur in the female patient."

In our clinic it has been customary to dilate the urethra with graduated Hegar dilators routinely as a part of the cystoscopic treatment of all female children with persistent urinary tract infection. This therapy has not been effective in all cases.

*Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

In September, 1950, Dr. Everett and one of us encountered a child of 5 with a complaint of "blood-staining, occasional pinkish urine, and stinging on voiding." Rectal examination revealed normal pelvic structures for a child of 5. The urine was cloudy and malodorous, and a gamma streptococcus was cultured from the bladder. Examination of the bladder through a No. 6 Kelly cystoscope revealed that the bladder was considerably larger than normal. There were no foreign bodies in the vagina. The child was started on Gantrisin* therapy, and one month later she had a cystoscopy with the No. 12 French miniature McCarthy cystoscope. There were moderate trabeculation and slight injection of the entire bladder mucosa. At the junction of the middle and anterior thirds of the urethra was a congenital valve, appearing as a transverse sheet of tissue attached to the posterior urethral wall. As the bladder was filled with fluid, this sheet floated anteriorly and almost completely obstructed the urethral lumen. The valve was divided with the miniature fulgurating wire. Following this procedure the child was continued on Gantrisin therapy, and the mother stated that except for slight bleeding one week after the operative procedure, she was asymptomatic. When last seen in May, 1951, the child was asymptomatic, the urine was clear microscopically, and the culture was sterile.

In September, 1952, we encountered a second patient, a 9-year-old child with a history of lifelong nocturnal enuresis, and partial diurnal incontinence as well for 2 years. The appetite was poor, she was frequently nauseated, and at times ran a low-grade fever. The urine was said always to have a strong odor. On examinaiton the child was pale and undernourished but quite cooperative. The general physical examination was essentially negative, but a catheterized specimen of urine contained many leukocytes and yielded coli-aerogenes on culture. The child was admitted to the hospital on the pediatric service for study. At cystoscopic examination on Nov. 14, 1952, the bladder appeared slightly injected with some reddening and bullous edema of the trigone. Both ureters were catheterized with No. 6 renal catheters. Sterile urine was obtained from both kidneys, and the plain plate and bilateral pyeloureterogram were normal. Examination of the urethra with the No. 14 French children's McCarthy cystoscope, however, revealed that the sphincter was irregular. A longitudinal fold, which floated out from the lateral urethral wall on either side, was described. These structures partly obstructed the lumen of the urethra. It seemed very questionable whether these structures could be responsible for the patient's symptoms, but on cystometric study there was residual urine of 270 c.c., after the child had voided 160 c.c. The cystometric curve was within normal limits. These findings were interpreted as indicating an obstructive lesion, and on November 21 the folds were resected with the No. 24 French McCarthy resectoscope, great care being taken to resect distal to the sphincter. A Foley retention catheter was kept in the bladder until the fifth postoperative day, and the child was kept on Gantrisin therapy. On discharge from the hospital on the eighth day, the urine was clear and the culture sterile. The mother reported in December, 1957, 5 years after the original treatment, that the child was well except for occasional nocturnal enuresis.

Since encountering these 2 cases we have been on the alert for similar urethral lesions in children who have persistent urinary symptoms, particularly those of recurrent infection. By December, 1957, we had collected a series of 25 cases of urethral obstruction. In 4 of these patients a diagnosis of transverse urethral valve was made, and in 21 the diagnosis was "longitudinal urethral fold."

Material

The present series consists of 25 children ranging in age from 17 months to 11 years whom we have treated during the years 1950-1957. The average age of the patients was 5 years and 1 month at the time of the first examination.

*Hoffmann-La Roche Inc., Nutley, N. J.

Symptoms

The duration of the symptoms of which our patients complained varied from 2 months to 9 years. In the 9-year-old child whose history is given above, symptoms had been present since early infancy. The average duration of the symptoms was 2 years. An analysis of these symptoms is shown in Table I. It will be noted that pyuria was most common and that fever, dysuria, and frequency of urination followed in that order. In most of the cases the symptoms were those of persistent or recurrent urinary tract infection, 24 of the 25 patients having either fever, pyuria, or a positive bladder culture. In 6 cases the mothers noted that the children had difficulty in voiding, and several volunteered the information that the children would "grunt" or "growl" when they tried to void. All the patients had had adequate conservative treatment before they were referred to us.

TABLE I. ORIGINAL SYMPTOMS

SYMPTOM	NO. PATIENTS
Pyuria (catheterized specimen)	19
Fever	16
Dysuria	13
Frequency	13
Enuresis	8
Difficulty in urination	6
Abdominal pain	6
Hematuria	2
Urgency	1

TABLE II. PREOPERATIVE CYSTOSCOPIC STUDY

FINDING	NO. PATIENTS
Trigonitis	11
Bullous edema of trigone	8
Trabeculation of bladder	7
Residual urine over 30 c.c.	6
Ureteral stricture, bilateral	1
Abnormal insertion of ureter	1
Hydroureter, unilateral	1
Hydroureter, bilateral	1
Hydronephrosis, bilateral	1
Hydronephrosis, unilateral	4

Urological Findings

As has been previously stated, 4 patients in our series had transverse urethral "valves," and 21 longitudinal "folds" which were thought to cause obstruction. All patients had had a complete medical workup including a neurological examination, and a complete cystoscopic study, including a bladder cystoscopy and retrograde or intravenous pyelograms. In order to rule out neurological disease cystometric studies have been done in most cases, and these have always been within normal limits. We consider a residual urine of 30 c.c. or less to be within normal limits in a child. Of our patients, 3 had a residual urine of 30 c.c. In 6 cases, it was more than 30 c.c., the largest amount being 270 c.c. and the average in these 6 cases 147 c.c. In the remaining cases the residual was less than 30 c.c. Our decision as to whether a urethral lesion caused obstruction was based primarily on the cystoscopic appearance of the lesion and the degree to which it appeared to obstruct the urethral lumen, but also on other factors such as residual urine, trabeculation

of the bladder, or evidence of obstruction of the upper urinary tract. The various other lesions which were discovered on cystoscopic investigation of the urinary tracts in addition to the urethral lesions are shown in Table II. It will be noted that trigonitis and cystoscopic evidence of cystitis were most common but that several patients had abnormal pyeloureterograms. The results of bacteriological study of the urines of our 25 patients are shown in Table III. Five patients had positive cultures for 3 different organisms, while 2 different organisms were cultured in 7 cases. It will be noted that coli-aerogenes was the commonest organism, and that various strains of streptococci were next most common. Many of the organisms were drug resistant.

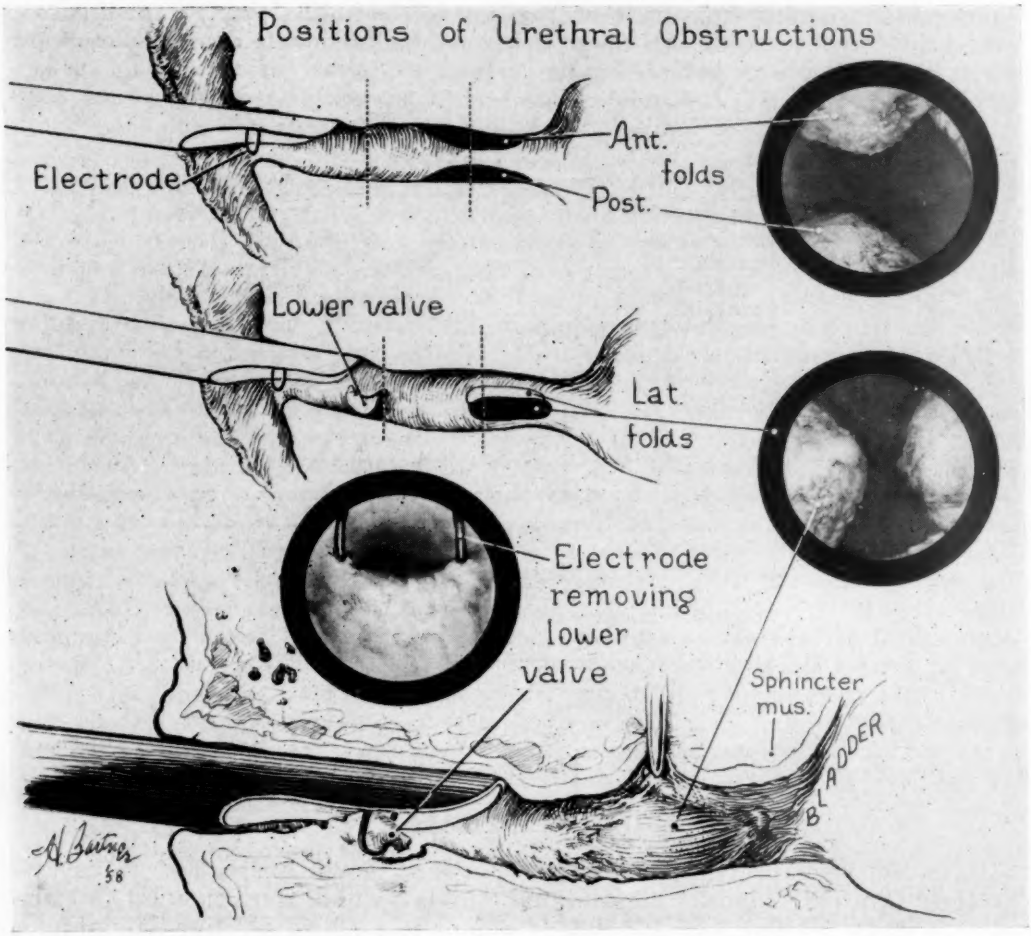

Fig. 1.—Positions of urethral obstructions, showing cystoscopic appearance and technique of resection. (×2.)

TABLE III. BACTERIOLOGICAL STUDY

ORGANISM	NO. PATIENTS
Coli-aerogenes	15
Streptococcus (alpha or gamma)	10
Proteus	9
Staphylococcus aureus	2
Pyocyaneus	2
None (sterile)	2

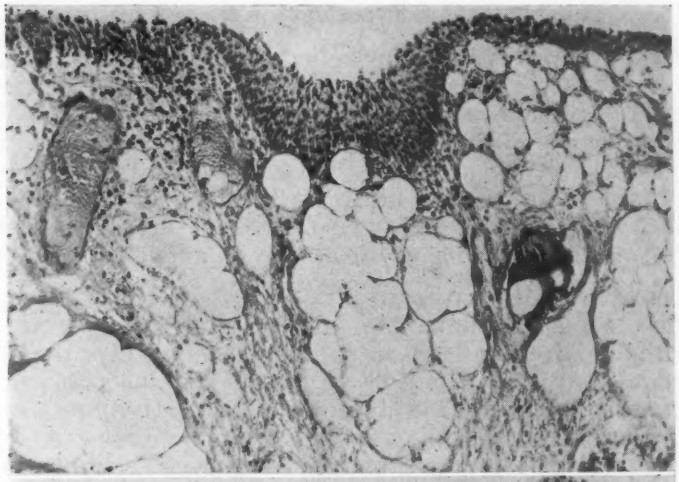

Fig. 2.

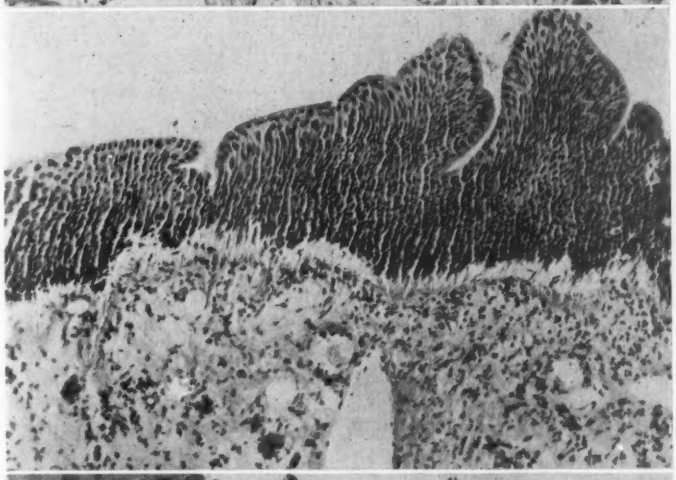

Fig. 3.

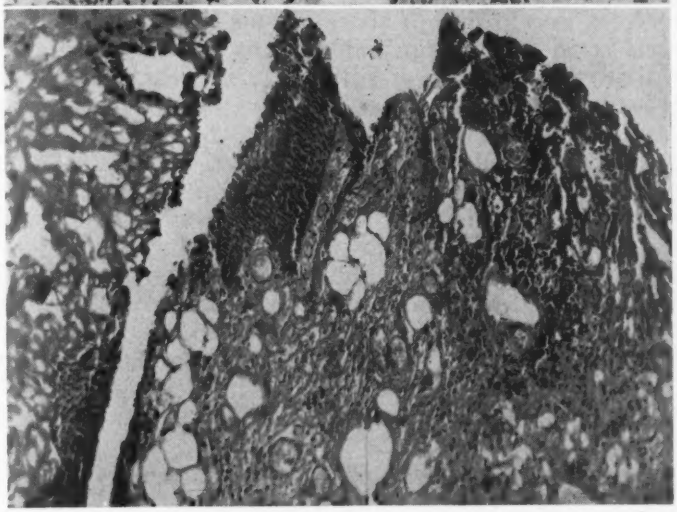

Fig. 4.

Fig. 2.—Resected urethral fold showing marked urethritis cystica. (×150; reduced 3/11.)

Fig. 3.—Resected fold showing epithelial hyperplasia, chronic inflammation, and urethritis cystica. (×150; reduced 3/11.)

Fig. 4.—Resected fold showing urethritis cystica and chronic inflammation. (×150; reduced 3/11.)

Treatment

As has already been stated, the valve or fold was destroyed by fulguration with the miniature electrode in 2 cases. In 23 cases, the lesions were resected. The adult resectoscope was used in one case and the No. 18 French children's resectoscope in 22. "Folds" are usually attached to the lateral urethral walls but may lie anteriorly or posteriorly. One to five folds may be present, but they are usually multiple. We have always been very careful to resect distal to the sphincter and to do most of the resection laterally, staying away from the posterior urethral wall as much as possible (Fig. 1).

It has been our routine to leave a Foley retention catheter, usually a No. 16 French, in the bladder until the fifth or sixth postoperative day. It is essential that the children be kept on suitable chemotherapeutic agents or antibiotics postoperatively until a sterile culture is obtained, and sometimes this takes many months. It is our routine to obtain sensitivity studies of the urine cultures in order to guide us in selecting a suitable drug. A routine culture is obtained one month after treatment, the patient is cystoscoped and the urethra dilated with Hegar dilators, usually to No. 7 or No. 8 at 3 month intervals for the first year, and at longer intervals thereafter.

Etiology and Pathology

The structures which we refer to as "valves" are thin, transverse sheets of tissue which arise from the posterior wall at about the junction of the middle and anterior thirds of the urethra. We believe that these are undoubtedly congenital. Microscopic study of the resected tissue in 3 of the 4 lesions of this variety shows connective tissue with edema and, in one case, chronic inflammation (Figs. 2, 3, and 4).

The second and more common type of lesion, we believe, has usually been described in the literature as a "polyp." These lesions, however, do not conform morphologically to a polyp. The lesion to which we refer as a "fold" appears to be a longitudinal structure which is oblong in cross-section and frequently extends from the posterior into the middle third of the urethra. There may be from one to five of these lesions but they are usually multiple. They have been considered "acquired" lesions by most writers, but the fact that we have discovered one in a child of 17 months, 2 in 2-year-olds, and 7 in 3-year-olds leads us to believe that many, if not all, of these are congenital. Microscopic study of tissue from 12 of the 21 "folds" showed that they were composed of connective tissue covered by urethral mucosa. Chronic inflammation was present in 7 and subacute inflammation in one. The most striking thing, however, is the fact that bullous edema or urethritis cystica was found in 10, and that in some cases it was very marked (Fig. 2). It is our belief that folds are usually congenital, but when urinary tract infection occurs, with its accompanying edema, the folds become larger and cause more obstruction. When there is residual urine, a vicious circle is established, and the urinary tract infection persists in spite of adequate chemo- or antibiotic therapy until the obstruction is relieved. It is interesting that in recent years Everett,[5] with one of us, has encountered 6 such lesions in adults.

Results of Treatment

The 25 patients whom we have treated for urethral obstruction have been followed for from 2 months to 5 years after therapy. The average duration of the follow-up was 21.4 months. The results of our follow-up studies are shown in Table IV. Fourteen of our patients, or 56 per cent, are completely

asymptomatic, and the remainder are improved. A sterile bladder culture was obtained at the last visit in 11, while in 3 others the culture showed only *Staphylococcus albus*, which was thought to be a contaminant. A second transurethral resection had to be done in one patient for folds which were not removed completely at the first operative procedure. This patient is now well 3 years after the second resection.

TABLE IV. RESULTS OF THERAPY 2 MONTHS TO 5 YEARS AFTER TREATMENT

	NO. PATIENTS	%
Asymptomatic	14	56
Improved	11	44
Unimproved	0	0
Bladder culture sterile	11	56
Bladder culture *Staphylococcus albus*	3	
Bladder culture positive for pathogens	11	44

One child still has a residual urine of approximately 60 c.c. following resection. Extensive bullous edema of the trigone is present, and we feel that she now has obstruction at the vesical neck. A careful resection of the vesical neck is planned for this patient in the near future.

The results of postoperative cystoscopic study of our patients are shown in Table V. It will be noted that the preoperative bullous edema disappeared after treatment in 4 of 8 patients, and preoperative trabeculation of the bladder wall in 4 of 7. In only one patient in whom trabeculation was originally absent did it appear following resection. This occurred in the child mentioned above, who still has significant residual urine, due, we believe, to obstruction at the vesical neck.

TABLE V. POSTOPERATIVE CYSTOSCOPIC FINDINGS

FINDING	NO. PATIENTS	
	BEFORE RESECTION	AFTER RESECTION
Bullous edema	8	4
Developed postoperative bullous edema		1
Trabeculation	7	3
Developed postoperative Trabeculation		1*
Residual urine over 30 c.c.	6	1*

*Both occurred in the same patient for whom resection of the vesical neck is planned.

Of the 5 patients in whom a diagnosis of hydronephrosis was originally made, 3 are known to have normal pyelograms following resection. One of these 3 children, however, was given postoperative ureteral dilatations for a stricture of the right ureter.

TABLE VI. POSTOPERATIVE SYMPTOMS IN 11 SYMPTOMATIC PATIENTS

SYMPTOM	NO. PATIENTS
Occasional enuresis	5
Recurrent cystitis	4
Frequency of urination	2
Pyuria	2

Of the patients who are still symptomatic, 5 have occasional nocturnal enuresis (Table VI). In only 2 is there persistent pyuria. Four still complain

of recurrent attacks of cystitis. In no case has a fistula or damage to the vesical sphincter resulted. In the patients in whom a urinary tract infection still persists after treatment, we believe it to be due in most cases to long established infection with a drug-resistant organism acquired prior to treatment.

Conclusions

1. Valves and folds causing urethral obstruction in female children are more common than is generally recognized.

2. A series of 25 such patients treated by transurethral fulguration or resection, of whom 14 are well and the remainder improved, is described.

3. A thorough search for obstructive urethral lesions should be made in all children with persistent urinary symptoms.

4. There is a place for the careful use of the resectoscope in the treatment of such children.

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Discussion

DR. EDWIN J. DECOSTA, Chicago, Ill.—Congenital urethral valves, as we thought we knew them up until this morning, have been generally considered to be due to transverse folds or diaphragms. These structures have been recognized for about 150 years and have been fully described. They have even been classified according to anatomic locations. They are exceedingly rare and exceedingly serious. By causing obstruction, transverse valves lead to severe renal damage and death. But this kind of obstruction is largely limited, rare as it is, to the male urethra.

Longitudinal folds too have been recognized, but these have been considered part of the normal anatomy of both the male and female urethra. In the female these folds are usually not prominent and have been thought to be clinically insignificant. The authors, however, have called our attention to possible significance of these structures as factors predisposing to and perpetuating bladder infection and causing urinary tract obstruction.

What is the relationship of abnormal urethral folds to polyps? It could just be that what the authors refer to as longitudinal folds have previously been called polyps, albeit such polyps would have a very long base.

I wonder, however, just where infection enters in. Is it really secondary to the obstructing fold or is it the cause of the fold? You will recall that 24 of the author's 25 patients manifested infection. On the other hand, in only 6 of 25 patients was obstruction sufficient to lead to retention or urinary tract dilatation.

In the last analysis it doesn't really matter whether these folds are congenital or acquired. What is important is that they interfere with free urinary drainage. Any structure which interferes with the free passage of urine is a potential cause of infection and back pressure. The importance of even minor degrees of urethral obstruction may be exemplified by Boyd's plea (Boyd, M. L.: J. A. M. A. 92: 2154, 1929) for meatotomy some 30 years ago. He pointed out the difficulty in eradicating infection in the female urinary tract if the meatus was narrowed. He also emphasized that the only complaints were those associated with infection. The patient was not mindful of any obstruction, not even the narrow stream, since comparison of the stream among females is most unusual.

Perhaps the problem with urethral folds is similar to that in other cases of mild obstruction. Their presence remains unknown until superimposed infection leads to edema and evident obstruction. Once they are recognized, however, it seems logical that removal alone offers promise of permanent cure. Dilatation provides only temporary relief; it will not remove the danger of partial and potential obstruction.

I would like to comment upon another aspect of this presentation, an aspect which is perhaps more philosophic than scientific. In the past, the attitude of members of the Department of Gynecology at Johns Hopkins was to condemn the use of the resectoscope for benign obstruction lesions in the female. Now, in 1958, Drs. Brack and Guild reflect the attitude of the same department by recommending resection of benign lesions of the urethra in female children. This indicates an alteration in thinking, a plasticity in receptiveness, and a willingness to adjust one's outlook if there is reason for change. It is only by such continual quest, intellectual curiosity, and bravery to accept change that we progress.

DR. BRACK (Closing).—I think these folds may have been referred to as polyps by others in the past, but I believe—in fact I am sure—that they are longitudinal structures rather than circular in cross section. I did not mention that it has been customary to dilate the urethras of these children with Hegar dilators for the first year after treatment so that we do not get contraction of the scar, and we have checked them at 6 month intervals after that. We thought we might be able to do some autopsy studies on children to learn more about the etiology of these lesions and we hope to report these findings some time in the future.

In closing I would again make a plea for the early diagnosis and treatment of these lesions in little girls.

URETHROVESICOPUBIC RELATIONSHIPS AND URINARY STRESS INCONTINENCE*

IV. The Uterine Suspension Syndrome

C. Paul Hodgkinson, M.D., and
William T. Kelly, M.D., Detroit, Mich.

(From the Department of Gynecology and Obstetrics, Henry Ford Hospital)

ANTERIOR displacement of the bladder, imprint of the uterus upon the posterosuperior surface, and partial elimination of the segment of bladder posterior to the urethrovesical junction, are three abnormal alterations in urethrovesicopubic relationships. They occur as the result of extravesical pressure. First observed after uterine suspension, these relationships have been noted to occur also in association with pelvic tumors. They stand out in contrast to the usual downward and backward rotatory changes incident to parturition. Urinary stress incontinence may accompany either type of change. It is the purpose of this report to evaluate the importance of the anatomic changes which occur as the result of posterosuperior extravesical pressure.

Material

Since 1949 the metallic bead chain technique[1] has been employed as part of the preoperative routine for patients with three types of clinical conditions: urinary stress incontinence, relaxation of support of the pelvic floor, and large intrapelvic tumors; over 500 cases have been investigated. For this presentation two groups of patients have been selected: Group I, 44 patients whose histories included uterine suspension; Group II, 42 patients who had not had uterine suspension, but whose radiograms showed anatomic relationships similar to those observed after this operation.

Normal Urethrovesicopubic Relationships (Fig. 1)

Previously established variations of normal urethrovesicopubic relationships served as the standards of reference.[2] In the normal nonparous patient the bladder was observed to be ovoid, centrally located, with the lowermost level midpubic. The urethra joined the base of the bladder at an oblique angle posterior to the midcoronal plane. The anterior third of the bladder occupied a suprapubic position.

The first changes effected by parturition were observed to result from inferior and posterior weaknesses; the suprapubic portion of the bladder tended to recede and the base of the bladder tended to sag. With uterovaginal prolapse the bladder rotated downward and backward; with urinary stress incontinence, vertical downward thrust without rotation occurred. The smooth bladder sil-

*Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

houette was replaced by one of irregular contour; the bladder wall appeared flabby. These urethrovesicopubic relationships were in agreement with the findings in similar studies reported by others.[3] Stevens and Smith[4] first reported on the metallic bead chain technique, and noted they had never found a normal bladder which extended anterior to the symphysis.

Relationships After Uterine Suspension (Figs. 2-5)

After uterine suspension the bladder is displaced anterior to the symphysis. This action involves chiefly the dome of the bladder, and any change in position of the base is more apparent than real. The segment of the bladder posterior to the urethrovesical junction is sharply reduced in size. Because of extravesical pressure on the posterosuperior surface, the normal shape of the bladder silhouette is altered to a rigid, somewhat angular shape showing the imprint of the constrained uterus.

Mobility of the bladder is minimal and the lower border is usually supported to a level of high normal.

Initially, the capacity is reduced. Gradually the bladder compensates by ballooning peripherally around the centrally impressed uterus. Radiographically these changes can be distinguished by variations in radiopacity.

Group I. History of Uterine Suspension.—

1. Clinical Appraisal.—By means of these anatomic changes, the 44 patients in Group I were divided into 3 classes:

Class A (typical suspension relationships): 27 patients whose urethrovesical relationships conformed in all respects to those known to follow uterine suspension;

Class B (absent suspension relationships): 11 patients whose relationships showed no influence from uterine suspension; and,

Class C (partial suspension relationships): 6 patients who showed at least one distinct characteristic of uterine suspension.

One additional patient on whom preoperative studies were not done is included because of relationships observed at the time of operation; she probably could have been included in Class B.

This intragroup classification provided means whereby symptoms could be compared with anatomic relationships.

2. Vital Statistics (Table I).—Distinct differences were evident for age, age at the time of the menopause, age at the time of the uterine suspension, and the number of patients who were delivered of infants after uterine suspension was performed.

TABLE I. VITAL STATISTICS, GROUP I

CLASS	AVERAGE AGE	MENOPAUSE		AVERAGE AGE UTERINE SUSPENSION	POSTSUSPENSION DELIVERIES (%)
		BEFORE (%)	AFTER (%)		
A	44	78	22	29	37
B	60	18	82	44 (37)	30 (60)
C	48	50	50	36	80

The average age of patients in Class A was 44 years; 22 per cent had involutional changes of the menopause; the age at which uterine suspension was performed averaged 29 years; and 63 per cent had all their children before the uterine suspension operation.

The average age of Class B patients (absent suspension relationships) was 60 years, 82 per cent were beyond the menopause; the average age at which uterine suspension was performed was 44 years; and only 30 per cent were delivered of an infant after uterine suspension. Critical review of the records of these patients suggested that the figures were distorted. Five patients of this class had ill-advised uterine suspension with the intention of correcting uterine prolapse at the respective ages of 41, 45, 61, 68, and 46 years. If these patients are excluded from statistical analysis of this class, the average age at which uterine suspension was performed was 37 years, and 60 per cent were delivered of an infant vaginally after that time.

These factors suggested that the effects of extravesical pressure caused by uterine suspension were mitigated by advancing age, involutional changes of the menopause, and vaginal delivery after uterine suspension.

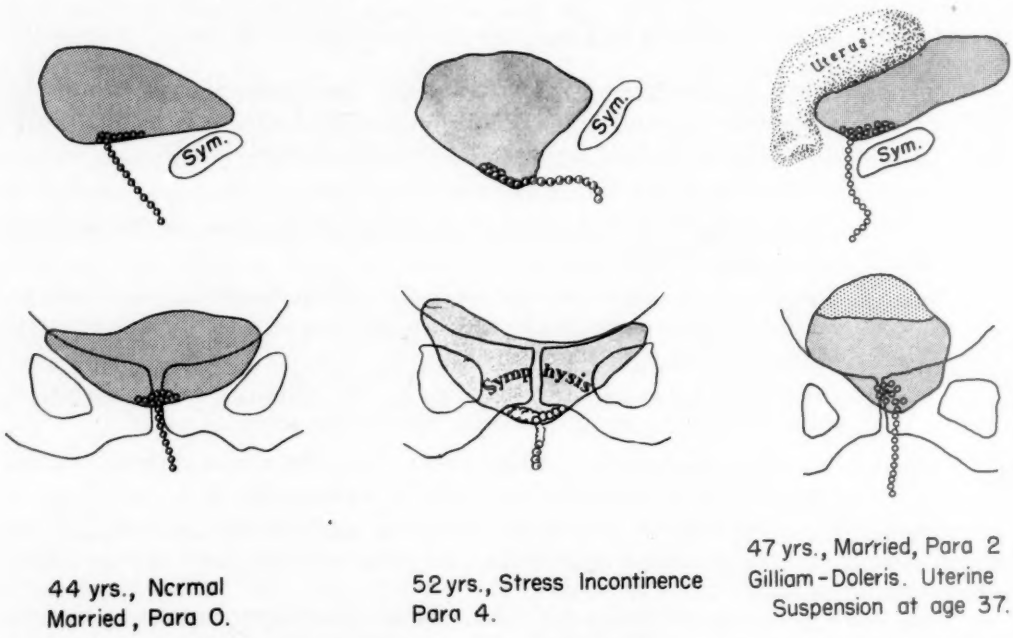

Fig. 1.—Comparison of urethrocystograms of the following types of patients: normal (left), with stress incontinence (middle), and after uterine suspension (right).

3. Symptoms (Table II).—Frequency, urgency, and stress incontinence were appraised according to the item count of incidence and the degree of severity. Multiple symptoms were usually present in an individual patient.

Urgency and frequency occurred in nearly all patients in Class A (typical suspension relationships); in several it was sufficiently severe as to be graded as "urge incontinence."

TABLE II. SYMPTOMS, GROUP I

CLASS	FREQUENCY DEGREE			URGENCY DEGREE			STRESS INCONTINENCE DEGREE		
	0	2+	4+	0	2+	4+	0	2+	4+
A	2	24	2	10	17	2	9	17	1
B	3	8	0*	5	6	0	8	3	0
C	0	6	0	2	4	0	1	5	0

*Four patients of Class B were unable to void unless they manually replaced their uterovaginal prolapse.

Stress incontinence was present in 18 (69 per cent) of the 26 patients of Class A. The degree of severity varied. In 17 patients it was considered to be of mild-to-moderate degrees, and in one patient it was extremely severe.

In contrast the patients in Class B (absent suspension relationships) had both a reduced incidence and a lessened severity of symptoms. Frequency, urgency, and stress incontinence were absent in 3, 5, and 8 patients, respectively. Four patients were unable to void unless they first manually replaced their uterovaginal prolapse. Three patients complained of mild stress incontinence.

4. Physical Findings (Table III).—Cystocele, urethrocele, rectocele, and uterovaginal prolapse were graded from 0 to 4 plus. In general, the patients in

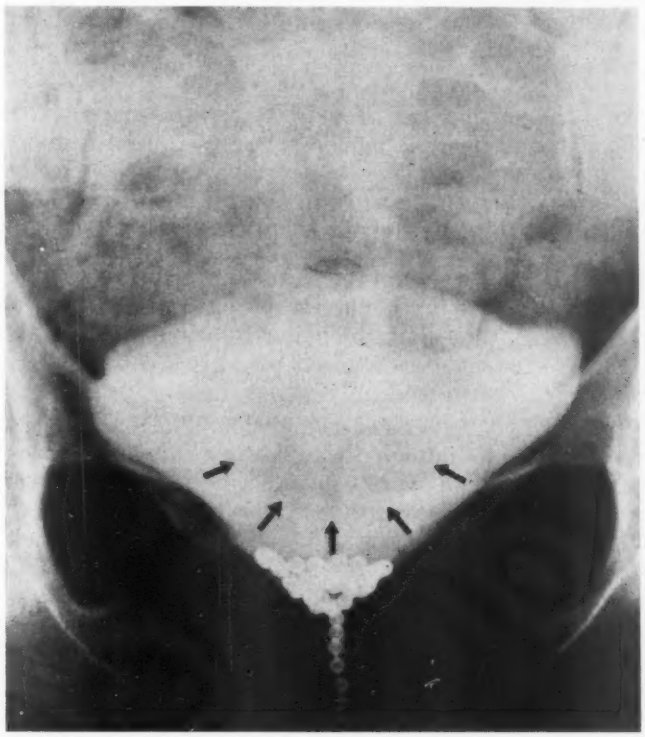

Fig. 2.—Photograph of anteroposterior radiogram showing the radiolucent shadow of the fundus of the uterus. Because details were difficult to demonstrate by photographic means, tracings have been used.

Class A had comparatively little loss of support while those in Class B experienced extensive degrees of relaxation. Respectively, cystocele, urethrocele, rectocele, and uterovaginal prolapse was absent in 6, 11, 13, and 21 patients in Class A; for patients in Class B (absent suspension relationships) the similar figures were 1, 1, 1, and 0.

Cystometric studies were performed upon 32 patients. The capacity of the bladder is shown in Table IV. While the number is too small to permit accurate induction, an important trend is indicated from the fact that 47 per cent of the patients in Class A had capacities of less than 350 c.c. On the other hand, while small capacity may have been detected as a trend, the ability of the bladder to compensate for the constraining pressure of the uterus is well demonstrated by the capacity in excess of 850 c.c. registered for 6 patients.

TABLE III. PHYSICAL FINDINGS, GROUP I

CLASS	CYSTOCELE DEGREE			URETHROCELE DEGREE			RECTOCELE DEGREE			PROLAPSE DEGREE		
	0	2+	4+	0	2+	4+	0	2+	4+	0	2+	4+
A	6	22	0	11	17	0	13	15	0	21	6	0
B	1	8	2	1	8	2	1	8	2	0	6	5
C	0	6	0	0	6	0	3	3	0	1	5	0

TABLE IV. BLADDER CAPACITY, GROUP I

CLASS	CAPACITY			
	350 C.C.	550 C.C.	850 C.C.	1,200 C.C.
A	9	4	5	1
B	1	2	3	2
C		1	2	2

In this study the mitigation of urinary symptoms in patients of Class B (absent suspension relationships) as compared to those of Class A (typical suspension relationships) was striking. Stress incontinence was not observed in any patient of Class B once full downward and backward rotation of the bladder developed. As the fixation of uterine suspension decreased, symptoms abated.

5. Operations.—In appraising the results of the operative procedures employed for the patients in the three classes, it was apparent that objectives varied. For patients in Class B (absent suspension relationships) the chief purpose of the operations was to correct uterovaginal prolapse; procedures for treatment of stress incontinence were employed only three times.

For patients in Class A (typical suspension relationships), the intent of the operative procedure was to improve or restore the physiologic function of the bladder. Twenty of the 29 patients in Classes A and C were operated upon primarily to relieve urinary symptoms. Usually the procedures were compounded to release the constraining influence of the suspended uterus, and to improve the retaining function of the sphincter mechanism. Most practically these were attained by combining abdominal hysterectomy and retropubic urethropexy, with either the technique of Marshall-Marchetti[7] or the round ligament procedure of Barns[8] and Hodgkinson and Kelly.[9] With vaginal hysterectomy, the procedures of Kelly or Kennedy were most advantageously employed.

Because the pressure influences of the suspended uterus could not be detected accurately by clinical examination, urethrocystograms were found to be essential for selection of the proper operative procedure. Vaginal plastic operations for the treatment of stress incontinence were ill chosen if urethrocystograms showed the urethra and bladder to be supported at a level of high normal.

6. Results.—Because patients in Class A (typical suspension relationships) were operated upon to restore or improve physiologic function, those of Class B (absent suspension relationships) to correct anatomic deficiency, and those of Class C (partially typical relationships) for both reasons, appraisal of results is complicated. If alterations of the anatomy toward normal are used as objective evidence of success, the results for patients in Class B included one failure and 10 acceptably improved. By subjective standards, no failure was recorded for the patients in Class B.

Different objective and subjective standards were necessary for evaluation of results in patients in Classes A and C. If one accepts the thesis that the constraining pressure by the suspended uterus is an inimical influence, then

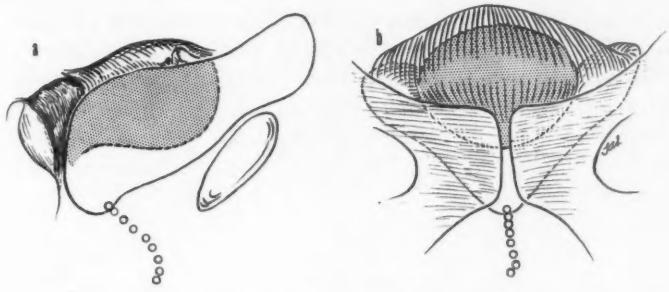

Fig. 3.—Impressionistic tracing of radiogram shown in Fig. 2. The uterus has been drawn in by Mr. Tom Stebbins to show the distortion of the anteroposterior surface of the bladder.

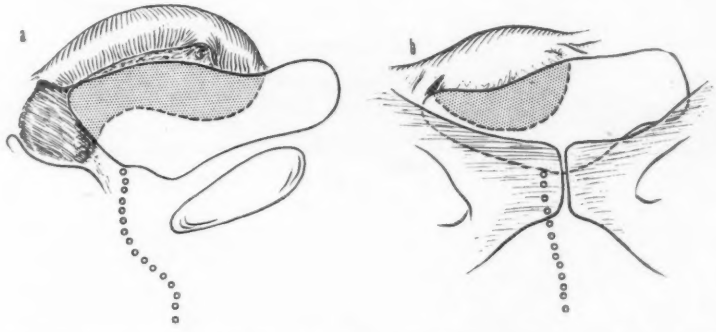

Fig. 4.—Impressionistic tracing to show the excellent support of the base of the bladder observed in some patients. The uterine fundus is partially superimposed over the superior surface of the bladder and displaced laterally.

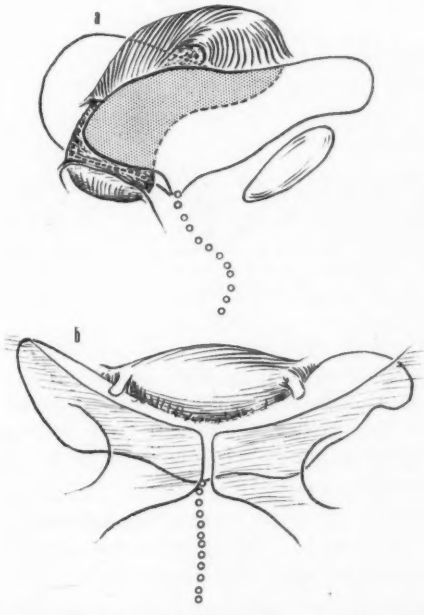

Fig. 5.—Impressionistic tracing showing complete superimposition of the uterus over the bladder, giving a "saddle" configuration to the superior surface of the bladder in the anteroposterior radiogram.

evidence that this influence has been lessened or removed as the result of operation is essential for evaluating postoperative improvement. To test fully this thesis, objective and subjective evidence must be correlated. Consequently, the rationale, in addition to the operative technique, needs to be tested.

Of the 27 patients in Class A (typical suspension relationships) and 6 in Class C (partial suspension relationships) all but 4 were operated upon. Counting 4 patients operated upon twice, 33 operations were performed upon 29 patients.

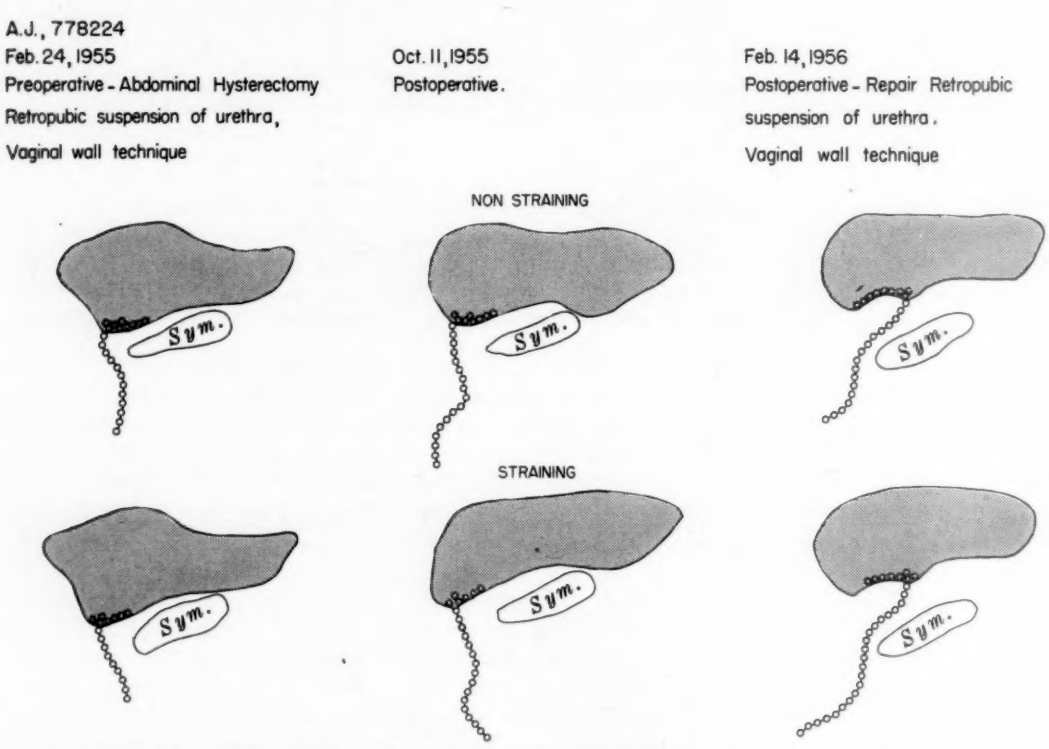

Fig. 6.—Operative failure. The urethrocystograms on the left show urethrovesicopubic relationships prior to abdominal total hysterectomy and retropubic urethropexy (Marshall-Marchetti technique). The urethrograms in the center show technical failure of the urethropexy. Reoperation to correct persistent urinary stress incontinence was performed one year later, and the radiograms on the right show the relationships following successful execution of the operative technique. These radiograms show that the bladder may be slow to recede after being disposed anteriorly by uterine suspension.

By the use of subjective standards, patients were declared cured if stress incontinence was relieved completely and if urinary urgency and frequency were intermittent and mild. If stress incontinence was completely relieved but if urinary urgency and frequency persisted to the degree that antispasmodics were required, the patient was declared improved. Failure was recorded if urinary stress incontinence persisted.

The patients in Classes A and C were considered collectively. With these standards, 14 (48 per cent) of the patients were cured, 10 (35 per cent) were improved, and 5 (17 per cent) were failures.

The pre- and postoperative urethrocystograms of the patients recorded as initial failures were compared. In each instance an anatomic reason for failure was detected. When this was corrected by reoperation, 4 of the 5 patients were cured; 1 has not submitted to reoperation.

Patient H. L. (Class C) suffered recurrence of stress incontinence 8 months after vaginal hysterectomy and cystourethroplasty (Kennedy technique). The preoperative urethrocystograms showed anterior displacement of the bladder, slight diminution in size of the posterior bladder segment and minimal descensus.

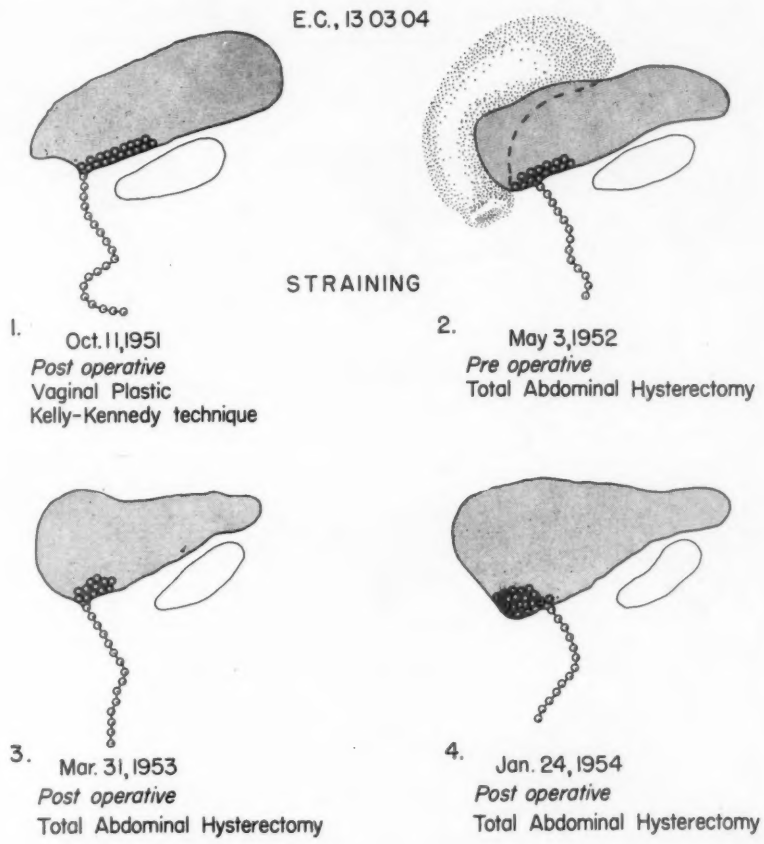

Fig. 7.—Operative failure. Radiograms *1* and *2* show urethrovesicopubic relationships after vaginoplasty with the Kennedy technique. Although restoration of bladder support was acceptable, stress incontinence persisted. After abdominal hysterectomy, expansion of the posterosuperior bladder segment is evident. Comparison of radiograms *3* and *4* shows the posterosuperior expansion to be progressive.

The relationships were typical of urinary stress incontinence in that the urethrovesical junction was displaced to the lowermost level of the bladder during straining. Although the bladder was supported to the normal level after operation, the relationships of stress incontinence persisted. After retropubic urethropexy (Marshall-Marchetti technique) she had full control of her urine and was declared cured.

Failure in this patient was considered to have been the result of improper selection of the initial operative procedure. Her experience serves as an example as to why, in patients with minimal cystourethrocele, vaginal plastic procedures frequently fail.

Patient A. J. (Class A) failed to obtain relief from urinary stress incontinence following hysterectomy and retropubic urethropexy (Marshall-Marchetti technique). Postoperative urethrocystograms showed no elevation of the urethrovesical junction, indicating that failure was caused from faulty technique. She submitted to the same operation one year later and was cured (Fig. 6).

Patient E. C. (Class A) was the victim of intractable urinary stress incontinence of 4 years' duration. The onset of her affliction was incident to suspension of the uterus by the Gilliam-Doleris technique. Urethroplasty (Kennedy technique) resulted in slight improvement. Postoperative urethrocystograms disclosed relationships typical of uterine suspension. Her bladder capacity was 300 c.c. Abdominal hysterectomy, performed to permit retrocession of the bladder and expansion of the posterosuperior segment, was followed by complete

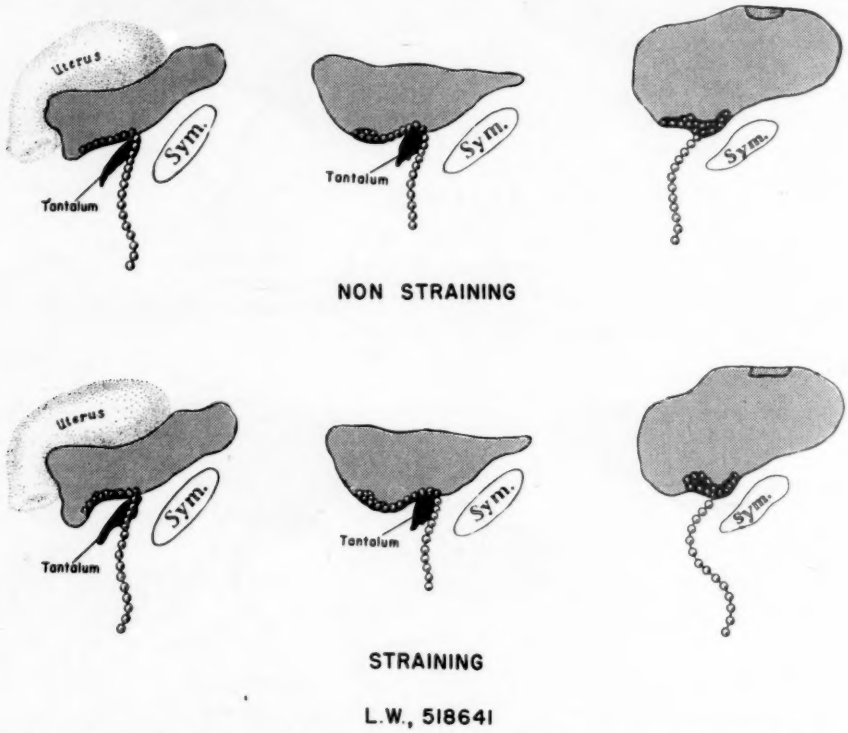

Fig. 8.—The postoperative radiograms show changes in urethrovesicopubic relationships effected by different operative procedures. On the left, the changes following retropubic urethropexy are shown. A previously placed tantalum bar inferior to the urethrovesical junction is shown. The center radiogram shows the changes following abdominal total hysterectomy. Because it was necessary to remove the tantalum bar, additional vaginal plastic operative work was required, and the subsequent urethrovesicopubic relationships are shown on the right. The patient is completely free of urinary stress incontinence.

cure. Because the significance of uterine suspension in the etiology of urinary stress incontinence was not initially appreciated, failure resulted. When these factors were recognized and, as the result of hysterectomy, dissipated, the patient was cured (Fig. 7).

Patient L. W. (Class A) was substantially improved following retropubic urethropexy (Marshall-Marchetti technique). Her bladder capacity was 150 c.c. Complete urinary control and normal bladder capacity were obtained by abdominal hysterectomy and subsequent vaginal urethroplasty. The latter operation was made necessary as the result of removing a tantalum bar, previously placed for suburethral support. The effects of hysterectomy were striking in this patient. Subsequently performed urethrocystograms showed the salutary changes in the position of the bladder to be progressive (Fig. 8).

B. K. (Class C), the fifth patient in whom failure was recorded, has not resubmitted to operation. Urethrocystograms performed following vaginal hysterectomy and cystourethroplasty show the relationships of stress incontinence.

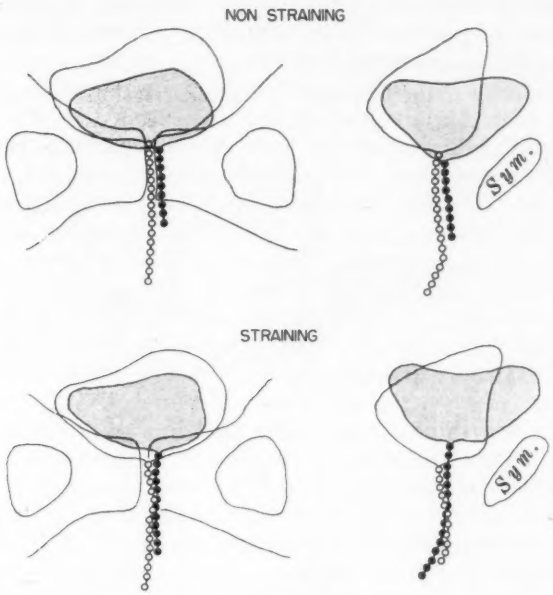

Fig. 9.—This patient, aged 31, para i, had numerous pelvic operations, including anterior suspension. Symptoms of urgency, frequency, and stress incontinence were severe. Bladder capacity was 200 c.c. The hatched bladder shadow and the black beads show the urethrovesical relationships prior to hysterectomy and release of intra-abdominal adhesions. The postoperative relationships are shown by the solid black line. Retrocession of bladder position and enlarged bladder capacity are shown.

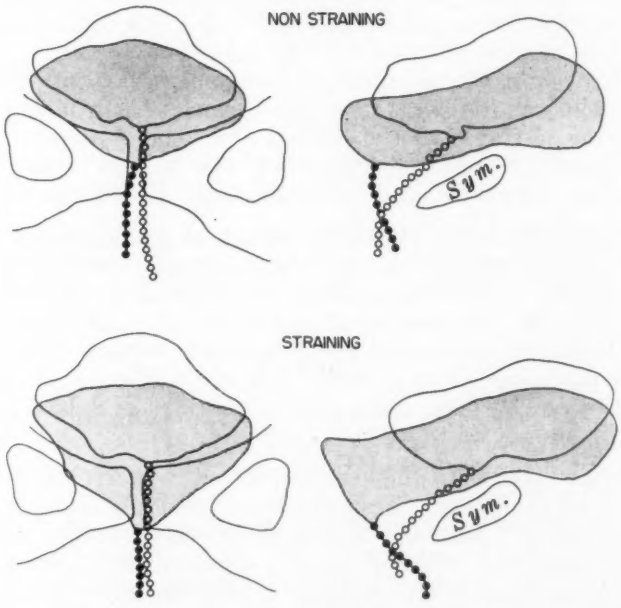

Fig. 10.—The relatively normal position of the bladder base makes clinical detection of these abnormal bladder relationships difficult. This position militates against successful repair from the vaginal approach, and, to some extent, prevents the establishment of optimum relationships by retropubic urethropexy. These radiograms show adequate elevation of the urethrovesical junction but unsatisfactory posterior bladder rotation.

Because the degree of cystourethrocele was minimal, the operative procedure was ill chosen; better results probably would have been obtained with abdominal hysterectomy and retropubic urethropexy.

Objective evidence of improvement was evaluated with the aid of urethrocystograms. Full technical improvement was accorded when the radiograms showed the constraining influence to have been removed; thereby the bladder was permitted to retrocede and the posterosuperior segment to expand.

In addition, the urethrocystograms permitted evaluation of the efficiency of the operation. Elevation of the urethrovesical junction above the lowermost level of the bladder is an essential feature of urethropexy; a sharp posterior angle is thereby created. In the vaginal plastic operations, the essential features are: (1) reconstitution of the vesical sphincter, (2) full support to the urethra, and (3) rational, but not excessive, correction of the cystocele.

Fig. 9 shows relationships considered to be favorable which resulted from hysterectomy alone. Simple release from the constraining influence of the suspended uterus did not immediately effect ultimate improvement. Follow-up urethrocystograms several months later showed improved changes in anatomy which were paralled by improvement in subjective symptoms.

The changes in urethrovesical relationships effected by combining retropubic suspension of the urethra with hysterectomy are depicted in Fig. 10. The high position of the bladder after uterine suspension tends to neutralize the effectiveness of elevation of the urethrovesical junction and posterior rotation of the bladder incident to retropubic urethropexy.

Group II. Extravesical Pressure Simulating Uterine Suspension.—

1. Clinical Appraisal.—When the urethrocystograms of 42 patients were scrutinized, relationships were discerned which simulated those of uterine suspension. The etiological factors were uterine myomas 34, adhesions from previous surgery 3, retrodisplacement of the uterus 3, congenital bands from the anterior cervix 1, and ovarian fibroma 1 (Fig. 11).

The average weight of the uteri and myomas was 250 grams. More important than weight was the position of the tumor. When located low on the anterior or posterior surface of the uterus, inimical pressure influences were likely. Third-degree retrodisplacement of the uterus when combined with adenomyosis, pelvic adhesions, and subinvolution was the mechanism in 3 cases.

2. Symptoms.—The symptoms resembled those observed in patients in Group I, Class A (typical suspension relationships); urinary urgency and frequency predominated. Only 16 patients complained of urinary stress incontinence. In all patients the degree of intensity of symptoms was mild. The capacity of the bladder, as revealed by cystometric studies, was as follows: less than 350 c.c. 4 patients (17 per cent); less than 550 c.c., 8 patients (33 per cent); less than 850 c.c., 9 patients (38 per cent); and less than 1200 c.c., 3 patients (12 per cent).

3. Operations.—The following operations were performed: (1) hysterectomy total abdominal, 9; (2) hysterectomy, total abdominal, combined with retropubic urethropexy (Marshall-Marchetti technique), 10; (3) hysterectomy, total abdominal, combined with retropubic urethropexy (round ligament technique), 3; (4) vaginal hysterectomy, 2; and, (5) vaginal hysterectomy combined with urethroplasty (Kelly or Kennedy technique), 10.

4. Results.—If failure to cure urinary stress incontinence and subsequent occurrence of stress incontinence are used as indices of results, then failure occurred three times, twice after total abdominal hysterectomy, and once after vaginal hysterectomy combined with urethroplasty.

If failure to obtain immediate relief from the symptoms of frequency and urgency is used to gauge the operative results for the patients of Group II, the cure rate is still less. Many patients showed gradual improvement over the subsequent 4 to 6 months, and, if given no additional treatment, were declared cured.

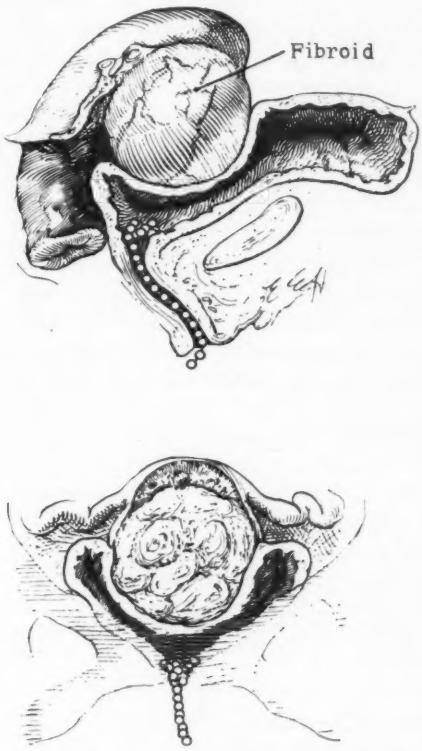

Fig. 11.—This impressionistic drawing shows uterine suspension syndrome relationships produced by a uterine fibromyoma.

If substantial alteration of urethrovesicopubic relationships toward normal is used as objective evidence of immediate operative success, the results were disappointing, because immediate shift of the position of the bladder frequently failed to develop.

Comment

The initiation or aggravation of urinary symptoms following uterine suspension, and their dissipation following hysterolytic procedures are cogent factors to support a direct cause-and-effect relationship for the "uterine suspension syndrome." How these changes of anterior displacement following uterine suspension compare with retrocessive changes following parturition is a clinical problem for contemplation. Harris, Mengert, and Plass[10] confirmed the observation of Halban and Tandler that the uterus was normally a mobile organ, and was subject to the dictates of gravity and contiguous pressure. Also, the bladder is highly mobile. Lynch[11] stated that the bladder is rarely disturbed by pressure and that it is able to distend in all directions. So long as the capacity is not curtailed, the organ is extremely tolerant to displacement.

The support for the base of the bladder in normal nonparous patients was shown to be afforded by the superior surface of the symphysis, the pubococcygeus,

muscles and the central pubocervical fascia.[2] With retrocession following parturition more responsibility for support came from the laterally placed pubococcygeus muscles and the pubocervical fascia of the levator hiatus.

After uterine suspension, support appears to come mainly from the superior surface of the symphysis. The lowest level of the bladder frequently is elevated to a position of high normal. These two factors—anterior displacement and high position—lessen the importance of the support usually afforded by the musculature of the pelvic floor; likewise, this operation adds to the rigidity of mechanical fixation.

Reference to extravesical pressure and urinary stress incontinence has been vague and relatively infrequent. Ullery[12] and Frost[13] mentioned inflammatory and neoplastic diseases of the uterus and rectum as possible etiological factors. Te Linde[14, 15] has repeatedly urged the gynecologist to broaden his professional purview to recognize secondary damage to the urinary system, possibly resulting from expanding pelvic masses. He cited the work of Everett and Sturgis in which they showed dilatation of the upper urinary tract in 50 per cent of patients with benign pelvic disease. Hundley and Diehl[6] reported similar results.

The evaluation and assignment of bladder symptoms in the female are extremely hazardous. Studies by Nemir and Middleton[16] and Kellar[17] showed that a high percentage of normal subjects admit having urinary stress incontinence. Nemir and Middleton questioned 1,327 young nulliparas. Urinary stress incontinence to some degree was admitted by 52.4 per cent. Laughing was the chief provocative factor. Kellar's study yielded similar results: of 134 healthy nulliparas, 87 admitted stress incontinence; 17 stated that it occurred frequently; less than half of the subjects admitted having urinary urgency. Although the degree of stress incontinence admitted by the subjects of these two groups probably should be considered to be of subclinical intensity, these studies suggested a natural propensity of a large percentage of human females to develop this annoying symptom.

Several patients of Class A stated categorically that their urinary symptoms were initiated by uterine suspension. For patient L. W., previously mentioned, the procedure started a chain of unsuccessful operations for stress incontinence which finally ended in ureterosigmoidostomy and, later colostomy because of chronic pyelonephritis. Chronic azotemia developed. Discouragement over her ill health contributed to marital discord and she was divorced. This stimulated the performance of another series of operative procedures which included retropubic urethropexy, excision of a previously placed suburethral tantalum bar, hysterectomy, and re-establishment of the continuity of the bowel and urinary systems. Now the patient is completely continent, and she has remarried.

In the patients of Class A (typical suspension relationships) frequency and urgency appeared to be more directly related to the anatomic changes induced by uterine suspension than did urinary stress incontinence. It appeared highly improbable that extravesical pressure per se could cause urinary stress incontinence. On the other hand, it appeared likely that potential stress incontinence would become overt in a patient subjected to uterine suspension should prior weakness of the sphincter-retaining mechanism be present.

Barnes[18] stated that since urinary incontinence, regardless of the exact etiology, represented a momentary increase in the forces of urinary expulsion over the powers of urethral resistance, it appears probable that incontinence could result from (a) an increase in intravesical pressure, (b) a weakening of the powers of resistance, or (c) a combination of the two.

Until the means of the voluntary control of urine are better understood, placing the prime responsibility in a phantom, physioanatomic sphincter-retaining mechanism appears the least objectionable. While the cinefluorographic studies of Ardran, Simmons, and Stewart[19] and Lund and associates[20]

have been most informative, certain facets of information are still not known. It is difficult to reconcile, on an anatomic basis, the return to the bladder of the urine in the proximal two thirds of the urethra upon voluntary inhibition of urination. Complete urinary continence has been observed many times following amputation of the female urethra. This indicates the presence of some sort of a retaining mechanism above the level of traumatic amputation. In addition, the part played by reversal of intra-abdominal pressure needs elucidation. Berglas and Rubin[21] showed that the pelvic cavity was subject to all the regulating changes of intra-abdominal pressure by the abdominal walls, and that the musculature of the floor of the pelvis was an intrinsic part of the physiologic complex of muscles. Lam[22] claimed that the intra-abdominal pressure was a relative figure. He showed that pressure measured at the uppermost level was registered as a negative quantity. When measured at the lowest level, the pressure was positive. While the interserous pressure was probably negative under all circumstances, the weight of the intra-abdominal contents negated this reading in the lowest levels.

For these reasons it is probable that uterine suspension and pelvic tumors play no more than ancillary roles in the development of urinary stress incontinence. Te Linde[15] warned against subjecting the patient with congenital retroversion to uterine suspension. He also stated that operative failure seldom occurred when the patient had a well-developed cystourethrocele. Failure was most frequently observed in patients without cystocele and in those with excessive scarring around the urethra as a result of previous operations. These warnings are especially applicable to patients with the uterine suspension syndrome.

Treatment

From experience with these two groups of patients, it is evident that great care must be used in selecting the proper operative procedure. Frank[5] warned "in the absence of cystocele and presence of stress incontinence, not to subject patients to operative procedures unless every psychical and neurogenic cause for the condition can be excluded." Because the anatomic relationships cannot be evaluated by clinical means alone, anteroposterior and lateral urethrocystograms should be employed. The high location of the bladder may be deceiving in the anatomic diagnosis of this type of urinary stress incontinence. It also militates against success if vaginal plastic operations of the Kelly or Kennedy type are employed. Not only is it difficult to accomplish technically a successful vaginal plastic operation under these circumstances, but nothing is done to alter the extravesical pressure of the uterus on the bladder. The admonition ". . . do a vaginal plastic procedure first . . ." does not hold for stress incontinence of this type. In our experience, the best operative procedures are those which combine hysterolysis and retropubic urethropexy.

Summary and Conclusions

Two groups of patients have been presented whose urethrocystopubic relationships showed anterior displacement of the bladder, reduction in the size of the posterior bladder segment, and evidence of posterosuperior extravesical pressure. Urinary frequency, urgency, and stress incontinence were the associated subjective complaints.

It appeared probable that these anatomic changes played no more than ancillary roles in the etiology of urinary stress incontinence. Yet, unless they were corrected, successful establishment of urinary control was in jeopardy.

Moreover, because of these peculiar urethrovesicopubic relationships, the surgical treatment must be carefully contemplated. Vaginal plastic operations are least likely to be successful. Abdominal procedures which involve hysterolysis and retropubic urethropexy are best.

Because the anatomic relationships are opposite to those usually observed in urinary stress incontinence, because they cannot be properly evaluated by clinical means alone, and because combined operative procedures must be employed if success is to be obtained, it has been found convenient to designate these objective signs and subjective symptoms, when occurring concomitantly, as the uterine suspension syndrome.

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Discussion

DR. RICHARD W. TE LINDE, Baltimore, Md.—Several points have been made by Dr. Hodgkinson which I believe should be emphasized: (1) the importance of a thorough examination of patients with incontinence before deciding on the proper therapeutic approach; (2) the conclusion that vaginal plastic procedures for stress incontinence are ill chosen if the urethra and bladder base are supported at a high level; and (3) the fact that failure to demonstrate elevation of the urethrovesical junction following the Marshall-Marchetti operation indicates deficiency of technique and clinical failure. I might add that the same is true of the sling type of operation with which I have had more personal experience.

So much for our points of agreement, but I must confess that I have not been completely convinced by the evidence presented of the "uterine suspension syndrome." I must admit, however, that we do not see in our area today many patients who have had uterine suspensions. Fifty years ago Dr. Kelly often suspended two or three uteri in a morning. Today, that is approximately the number of operations which are done primarily for uterine suspension in a year in our clinic where we do over 3,000 operations. Over the past years, however, I have seen many patients after uterine suspension and have not been impressed with the association of urge or stress incontinence in these patients. A properly done suspension should put the uterus in the position in which it normally was meant to be. This

being the case, it is difficult for me to visualize the mechanics of urge or stress incontinence when the uterus has been restored to its normal position. On the other hand, many uteri have in the past been erroneously suspended for these bladder symptoms. I can find nothing in the present study to indicate what symptoms these patients had before the suspension was done. Since many of the suspension operations were done many years before, it would be almost impossible to get this information. Is it not possible that in some of these patients the symptoms preceded the suspension and were persistent after the operation? Also included among the patients with the "uterine suspension syndrome" are some who had children after the suspension and developed cystourethroceles which, in my opinion, would be more apt to account for the urinary symptoms than the suspension operation.

Although postsuspension patients are not very frequent in our area, Negro women with large fibroids are. Stress incontinence and urgency depend not only upon the strength of the vesical sphincter, but also upon the intravesical pressure. The latter may be increased by excessive obesity and intra-abdominal tumors. Such large space-occupying tumors may be a factor in frequency and stress incontinence. On the other hand, moderate-sized fibroids seldom give rise to these symptoms and it seems that they should, if the replacement of the normal-sized uterus to its proper position is capable of causing such symptoms.

I mention these objections to emphasize how cautious one must be in evaluating cause and effect when considering a clinical syndrome. I honestly believe that much more work must be done before we can accept the "uterine suspension syndrome" as a clinical entity. I do appreciate, however, the great amount of work such a study entails and this presentation has alerted me so that in the future I shall be watchful for evidence of this syndrome. I prefer at this time to retain an open mind.

DR. LAWRENCE R. WHARTON, Baltimore, Md.—I find it rather difficult to discuss this paper today because we see so few uterine suspensions and we do so many less than formerly. I do not believe I could assemble a group of 50 patients after suspension in many years of practice, so that I feel this is a condition we do not see enough of in Baltimore to evaluate from this viewpoint.

I think we are indebted to Dr. Hodgkinson for one thing, however. We have always felt that uterine suspension was an operation that should not be done without great consideration and as he adds one more reason why we should no do it we are grateful to him.

I would like to discuss, as Dr. Te Linde did, the interpretation of one feature of the syndrome on which is based the indication for hysterectomy in women who have stress incontinence. I refer to what Dr. Hodgkinson calls the imprint of the uterus on the bladder. We do a great many cystograms and intravenous pyelograms in our studies and we very frequently see a concavity in the cystogram caused by the pressure of the uterus lying above the bladder. So far as I know, our x-ray department has not paid much attention to this picture and I myself do not lay much stress on the fact that the normal uterus lying above the bladder can produce this change in the outline of the bladder. I do not remember any case in my own practice in which I thought the pressure of the normal uterus on the bladder was producing bladder symptoms. We have, however, seen patients who have had hysterectomy for various urinary symptoms and always without benefit. I do not want to criticize Dr. Hodgkinson's conclusions on this but in discussing his conclusions I feel that we should know more about these individual cases in which hysterectomy was done because of pressure by a normal uterus. I feel that it would be unfortunate if the general surgeon were allowed to get the impression that a cystogram which showed pressure of a uterus on top of the bladder in a woman with stress incontinence was justification for hysterectomy. The members of this Society have long tried to bring to the attention of doctors the fact that hysterectomy should be done only for definite pathological indications. After we know more about Dr. Hodgkinson's work and the cases he has studied we may be able to modify what I have said, but I would be slow in doing hysterectomy in these cases.

Finally, I would like to discuss one of Dr. Hodgkinson's comments, that stress incontinence is due to either (1) weakness of the sphincter muscle or (2) increase in the intravesical pressure caused by one of many ancillary factors. With this, I am completely in

accord. When viewed in this general light, we may divide the problems of stress incontinence into three parts. First, cases in which the urinary sphincter is normally strong and efficient. These women never have stress incontinence no matter what ancillary factors they may develop, uterine fibroids, prolapse of the uterus, pregnancy, psychoneurotic instability, or any one of many other conditions. Then, at the opposite extreme, are found those in whom the sphincter mechanism has been severely damaged, perhaps by a transurethral resection. These women are incontinent and do not need any ancillary factor to produce it.

Then, finally, there is the large group of women whose sphincter mechanisms are not quite perfect but who have stress incontinence only when some ancillary factor adds something to the intravesical pressure or lessens the efficiency of the sphincter which is already below par. The great majority of our cases of stress incontinence belong to this group, and as we can see at a glance, most of these ancillary factors are basically medical problems. Likewise, we have found that the strengthening of the sphincter mechanism often can also be accomplished without surgical intervention. This is therefore a situation in which it is extremely important to study the patient as a whole before embarking on surgery. In some of these women there is present a simple thing like menstruation or a psychoneurotic disturbance or the fact that she is too fat or has a urologic condition which makes it impossible for the urinary system to work properly—all these things must be investigated in studying a case of stress incontinence. Most of them are medical situations requiring careful medical and sympathetic care. I would also suggest that in many of these cases, excepting the one with prolapse or a large fibroid or injury to the urethra, medical care will eliminate many factors which influence and precipitate stress incontinence, through the use of exercise. This sort of treatment, carefully done, will in the long run produce a great many cures and will eliminate many people who will never need operation and finally will screen out cases in which it is perfectly clear that operation is necessary. I do not know whether Dr. Hodgkinson has used this method of screening. I know his paper was strictly a surgical discussion, but I think it is a good thing, even in presenting such a paper, to indicate to the general reader, who may not be as well versed as this group, that it is necessary in these cases to find some reason for operation and to eliminate the cases in which the operation is not necessary.

DR. JOSHUA WILLIAM DAVIES, New York, N. Y.—Dr. J. Wesley Boveet, of George Washington University, was interested in the cervical supports of the uterus, so he devised several operations which were destined to restore the cervix to its normal position in the midpelvis. He believed that the tonicity of the uterosacral ligaments assisted in maintaining a flattened bladder trigone. It may seem curious that a full bladder does not compress the walls of the vagina and bulge into the concavity of the sacrum. It is the tough fibrous capsule of the trigonal area which prevents such displacement. The trigone is attached to the lateral pelvic wall through connective tissue and inferior vesical blood vessels. Its proximodistal flattening is due to the tonicity of the uterosacral ligaments. In the erect posture the plane of the trigone is practically vertical. As the bladder fills with urine that portion of the muscularis which extends from the urethra to the urachus rests against the urethra and tends to compress it as intra-abdominal pressure is increased. If the fundus of the uterus is attached to the anterior abdominal wall the cervix is displaced and the bladder trigonal area relaxes and becomes congested. The uterine suspension syndrome referred to by Dr. Hodgkinson may be the result of trigonal relaxation and urethral congestion. The treatment for such urinary complaints is preventive. The uterus should not be fixed to the anterior abdominal wall.

Occasionally it is necessary to replace a retroverted uterus because of symptoms; but before the attempt to restore the uterus to an anterior position, it is necessary to separate adnexal adhesions which are responsible for holding the fundus posteriorly. *The Journal of the American Medical Association,* vol. 154, pp. 749-751, describes an additional adhesion between the sigmoid colon and the left round ligament which may be responsible for a torsion and a retroversion of the fundus of the uterus. If such an adhesion is divided, the smooth muscle in the round ligaments will be observed to contract and by so doing to

draw the uterus away from the raw adhesion area. Should it be necessary to draw the uterus away from divided adhesions, it is advisable to attach the round ligament in the region of the internal abdominal ring.

DR. JOHN B. MONTGOMERY, Philadelphia, Pa.—I am in agreement with Dr. Te Linde that bladder symptoms and certainly stress incontinence following uterine suspension are most unusual. This operation is not frequently done nowadays. In 1942 we investigated 500 patients who had been operated upon by the Simpson technique during the previous 20 years. Persistent bladder symptoms were unusual in that group of women.

I do not believe that Dr. Hodgkinson intended to convey the impression that distressing bladder symptoms frequently result from uterine suspension. He has presented a selected group of patients in whom stress incontinence developed following this procedure. It would be interesting to know what type of suspension had been done. I would like to know also whether Dr. Hodgkinson noted unusual displacement of the bladder or any other associated lesion that might have been responsible for the incontinence.

DR. HODGKINSON (Closing).—The discussants have rightfully emphasized the controversial place these anatomic changes have in gynecologic surgery for urinary stress incontinence. This paper, however, was not intended to be a criticism or an evaluation of the operation of uterine suspension. It was intended to be an appraisal of the importance of the changes in the anatomy of the bladder incident to uterine suspension and pelvic tumors. Certain types of patients have a propensity for developing three anatomic changes. For instance, the patient with the congenital retroversion of the uterus, in whom the anterior vaginal wall is short and in whom the cervix is located forward on the anterior vaginal wall is prone to develop these relationships with suspension of the uterus by the Gilliam-Doleris technique. In reoperating upon these patients, we observed that the ones most likely to show these relationships were those in whom the operation had been technically successful. Frequently, the uterine fundus was found to be densely adherent to the anterior abdominal wall. That not all patients who have had suspension of the uterus develop urinary symptoms was illustrated by the patients in Group I, Class B; neither did they show the typical relationships in anatomy known to follow suspension of the uterus.

Dr. Wharton emphasized the importance of employing, preoperatively, conservative means for management of the patient with urinary stress incontinence. We routinely employ perineal exercises and also find them to be of value in the postoperative period. The patients included in this report represent but a small percentage of the over 500 patients observed and it was not our intention to detail the over-all treatment of urinary stress incontinence. It appeared to us that this type of urinary stress incontinence was on a different anatomic basis from that usually observed.

I would like to reaffirm one impression implied by Dr. Davies concerning any change we made in the position of the pubic bone. We have not changed the position of the pubic bone. These slides are unaltered tracings of x-rays obtained with the patient in the erect position.

The comments by Dr. Montgomery were most apt. I have read the paper by Drs. Anspach and Montgomery on suspension of the uterus by the Simpson technique. I believe it was the last paper on suspension of the uterus presented before this society.

OVARIAN HOMOGRAFTS IN THE PRIMATE: EXPERIENCE WITH MILLIPORE FILTER CHAMBERS* †

SOMERS H. STURGIS, M.D., AND HECTOR CASTELLANOS, M.D., BOSTON, MASS.

WITH THE TECHNICAL ASSISTANCE OF JOHN RAHILLY

(From the Departments of Surgery and Gynecology, Harvard Medical School and Peter Bent Brigham Hospital)

IT IS quite possible that the next dramatic phase in the saga of clinical surgery may be characterized by the achievement of functioning transplants of tissues or organs from one individual to another. Except between identical twins and in a few isolated examples of patients with unusual pathological conditions, it has not as yet been possible to overcome the destructive antigenic reactions that prevent survival of homografts in the human. In the laboratory, homo- and heterotransplants to the anterior chamber of the eye or the hamster cheek pouch have survived when they have become vascularized.[1, 2] Even then, their function has been inadequately demonstrated.[3] Algire and associates[4] have made extensive use of the Millipore filter membrane in studying the principles underlying immune reactions induced by tissue transplants. These membranes exclude all circulating cellular elements, yet the pores are large enough to allow diffusion of proteins and electrolytes sufficient to nourish the enclosed tissue. They showed that nonvascularized homotransplants could survive without destruction for indefinite periods when enclosed in such a filter chamber. In their earlier work they used benign transplants (Harderian gland) as well as malignant tissue.

It occurred to us that a practical clinical application of this important work might appropriately lie in the field of endocrine organ transplantation. We chose to study transplants of the normal ovary within filter chambers constructed similarly to those of Algire to test whether function as well as survival could be achieved. Successful preliminary results in rats and monkeys have been recorded from this laboratory.[5, 6] This report summarizes our present techniques and records our initial attempts to obtain function in three nonvascularized human ovarian homotransplants. A discussion of the particular problems involved in applying such laboratory methods to clinical material is included.

*Supported in part by the American Cancer Society Institutional Grant as well as the William F. Milton Fund at Harvard University.

†Presented at the Eighty-first Annual Meeting of the American Gynecological Society, Asheville, N. C., May 19-21, 1958.

Technique

A. Filters.—Millipore filters* are semipermeable membranes of cellulose ester filaments constructed with a predetermined and uniform pore size. Preliminary studies had shown that an "HA" filter with pore size of 0.45μ would allow passage of gonadotropins but exclude leukocytes and red cells,[1] whereas a pore size of 0.8μ ("AA") allowed for penetration of cellular elements. The original membranes were relatively fragile. In the experiments reported here we have used HA filters within the substance of which has been incorporated a fine nylon mesh ("HAN").

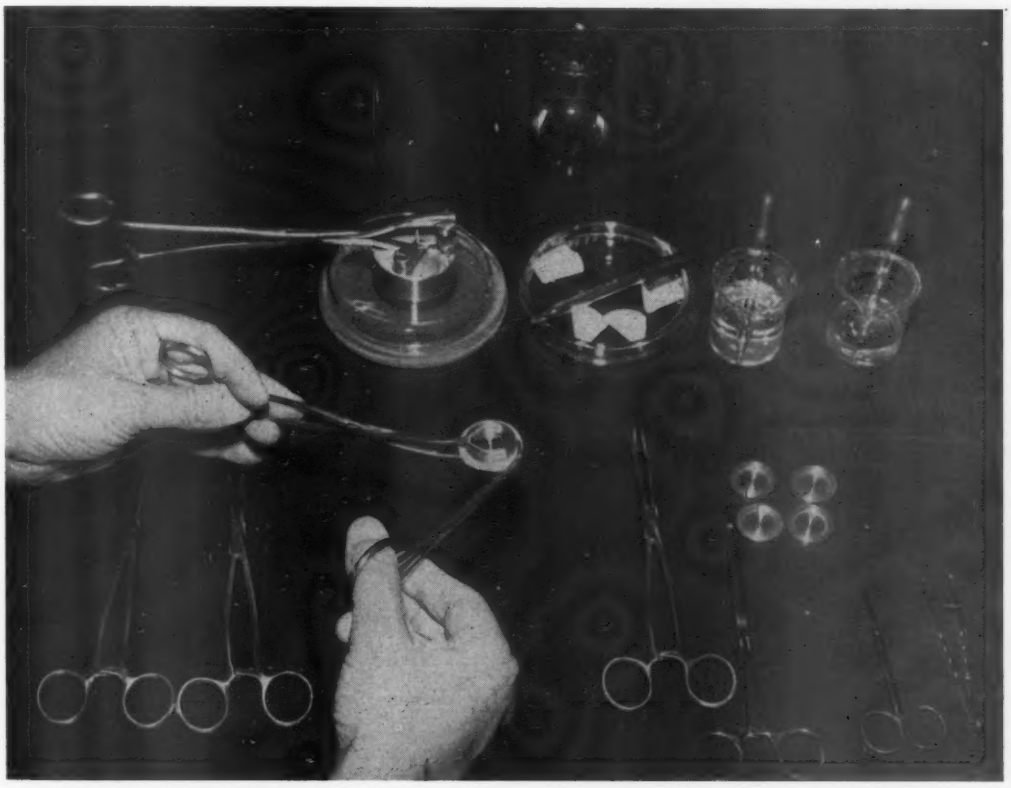

Fig. 1.—Procedure for cementing filters together. Steel disks, hollowed on one side, are centered above and below two 1 inch diameter Millipore membranes in special clamp seen above at left. The protruding edges of the membranes can then be cemented together as seen below without danger that the cement will infiltrate within the chamber.

B. Operative Procedure.—We have gained the impression from experience in rats that the shortest time after removal of donor tissue to implantation in the recipient distinctly favors survival of the transplant. Therefore in primate operations three teams are required. The donor team exposes and takes an ovarian biopsy, passes it to the team in the operating room for incorporation in multiple chambers under sterile conditions, while the recipient team in the next room simultaneously prepares the bed for the chambers. Generally not one but several small biopsies are taken from the donor in sequence to cut down further the time of passage from donor to recipient.

*Millipore filter membranes were obtained from the Millipore Filter Corp., Watertown, Mass.

C. Preparation of Chambers.—Circular wafers of HAN, with a diameter of 1 inch, are previously sterilized in ethylene oxide gas. They are soaked in sterile Hanks' solution in the operating room. Donor tissue is minced as finely as possible with iris scissors and twenty to forty small fragments centered in one wafer. A second wafer is superimposed. We have discarded use of Lucite rings as developed by Algire, which are no longer necessary to support the tough nylon-impregnated membranes, and decidedly too bulky for multiple implants. The problem of gluing together the edges of the two membranes without infiltration of the cementing substance into the interior has been met

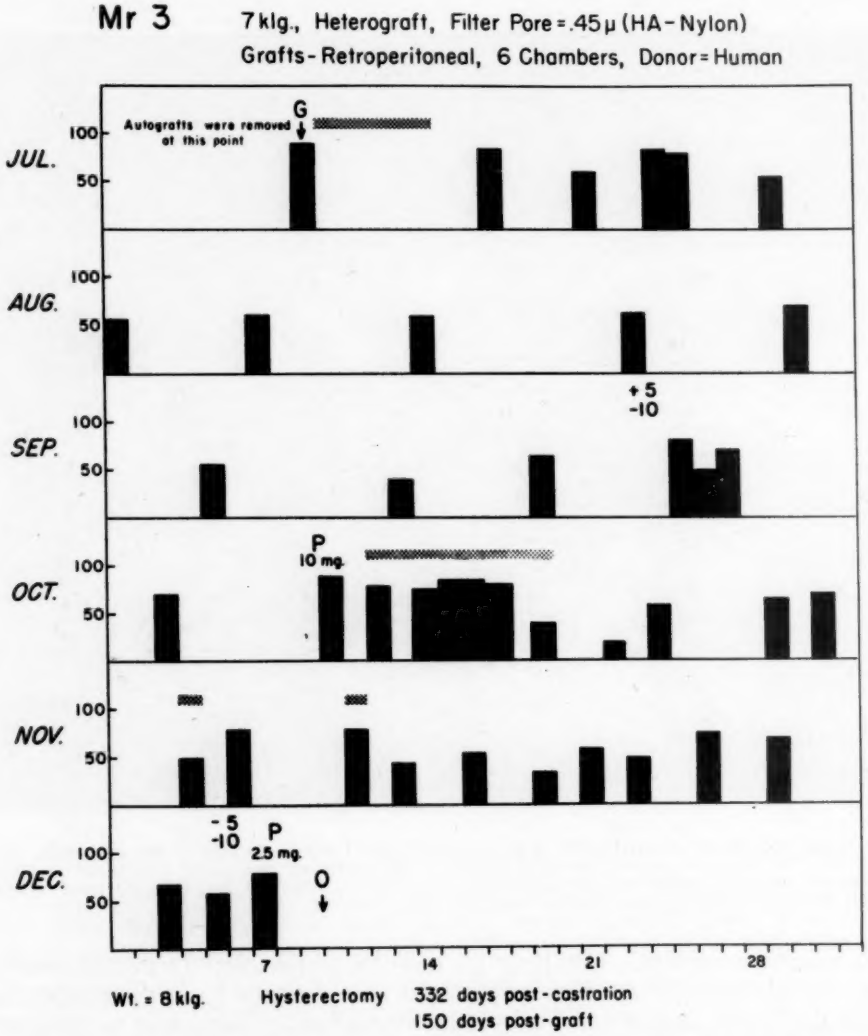

Fig. 2.—Heterograft of human ovary tissue to *Macacus rhesus*. Ovarian autotransplants were removed at time of graft (*G*). Cross-hatching indicates vaginal bleeding. Per cent of cornified cells in vaginal smear represented in black vertical columns. Figures refer to FSH determinations in mouse units (*+5*, *−10*). *P*, Progesterone in oil given intramuscularly. *O* represents operative removal of grafts.

Fig. 3.—Endometrium of Macacus 5 months after heterograft of human ovary fragments in a Millipore chamber.

Fig. 4.—Same as Fig. 3, high power.

Fig. 5.—Cells from within Millipore chamber 5 months after heterograft of human ovary in monkey.

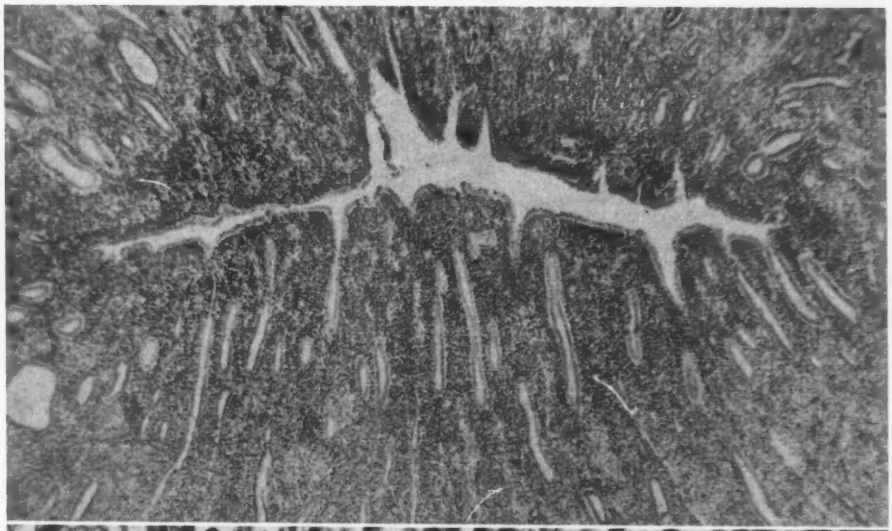

Fig. 3.

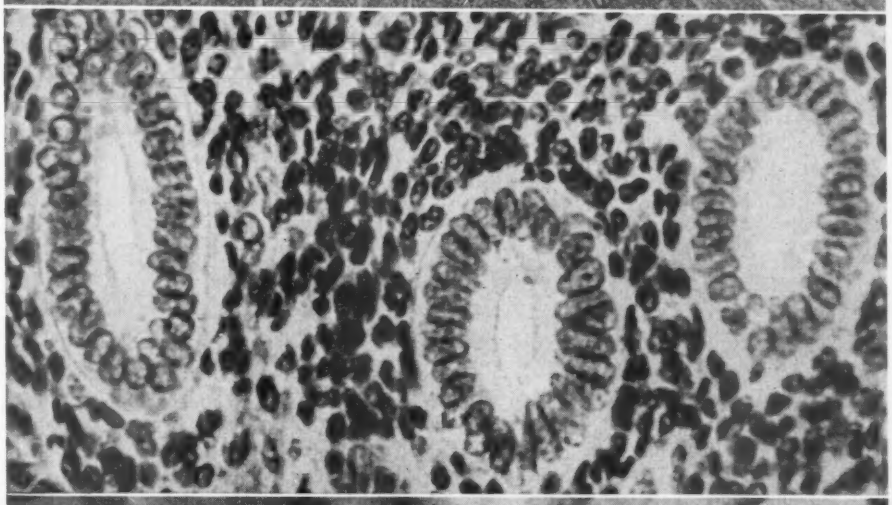

Fig. 4.

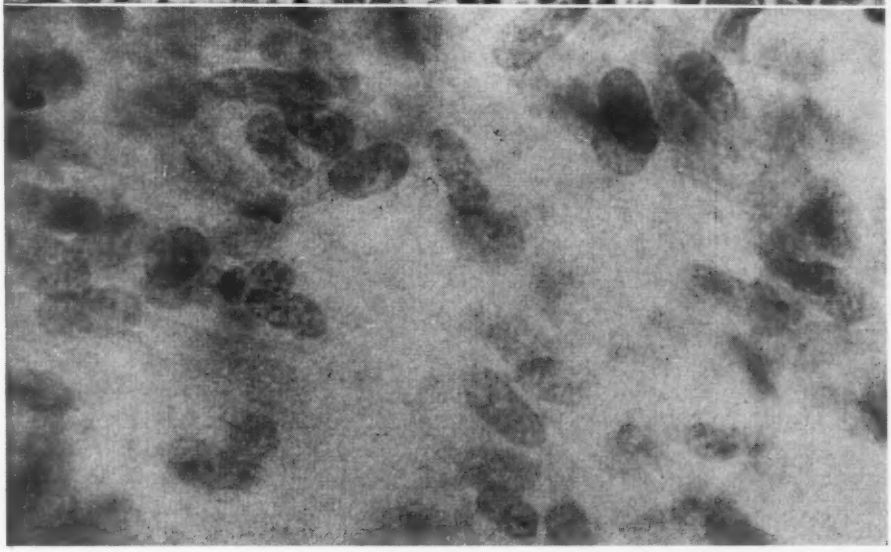

Fig. 5.

Figs. 3-5.—*For legends see opposite page.*

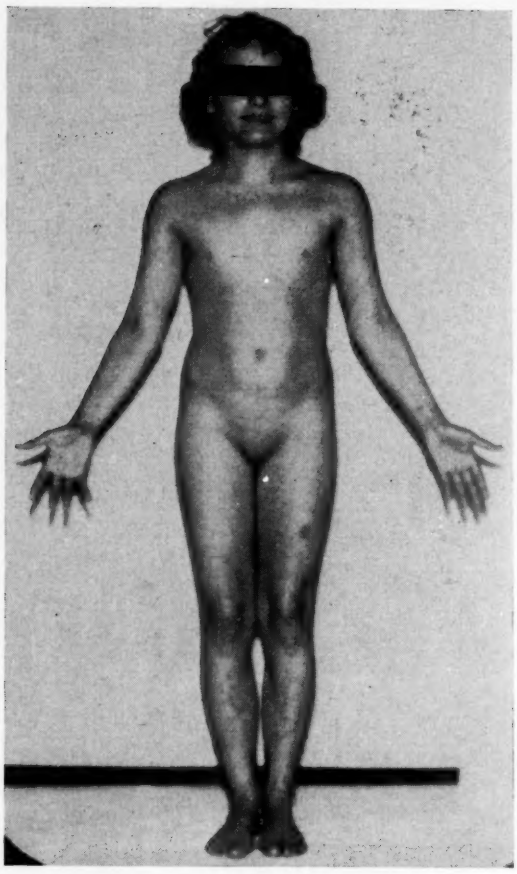

Fig. 6.—Case 9K768.

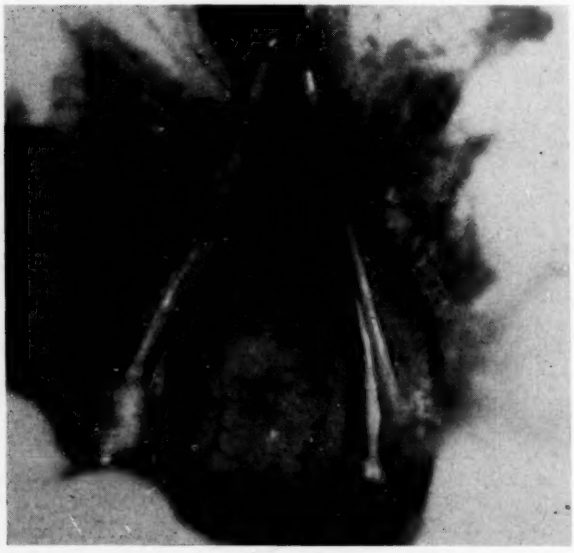

Fig. 7.—Pelvic findings in Case 9K768 showing absence of uterus and typical linear rudiments of ovaries only.

by construction of finely tooled steel disks, hollowed on one side. These are centered, one below and the other above the loaded double membrane, and clamped tightly together with a special clamp (Fig. 1). The central cavity prevents crushing of the tissue fragments, but the rims are held in such tight apposition that no significant infiltration of cementing substance can occur. The disks are slightly smaller in diameter than the 1 inch wafers. The overlapping edges can then be glued together, the clamps released, the steel disks removed, and the chamber is then ready to imbed. Since the membranes are soluble in acetone, a special cement was developed of Millipore-saturated acetone. This is painted on the free edge of the double membrane, the edges pressed together, and where it dries, within 3 minutes, a tight weld of the two surfaces occurs. It takes approximately 8 minutes to prepare a chamber. At conclusion of the operation, an extra chamber is loaded and the wet weight of tissue in this sample is calculated by comparing its weight with that of another double chamber similarly prepared but without any tissue.

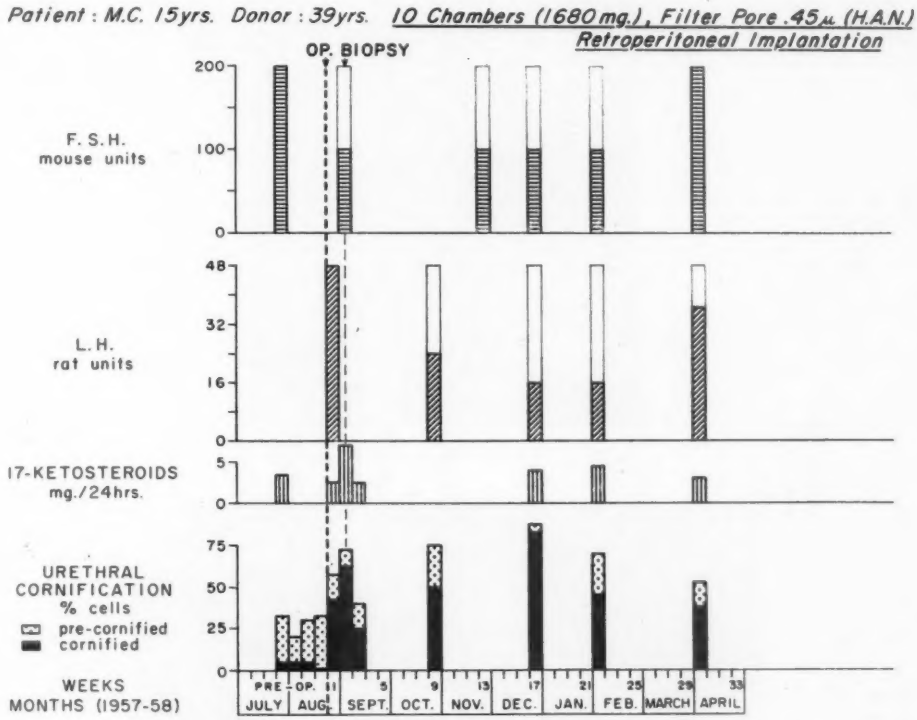

Fig. 8.

D. Choice of Donor Tissue.—In preliminary experiments (to be reported) it seemed clear that persistence of function was related to cell type. Minces of ovarian stroma and follicles functioned well in the rat, whereas, when only ovarian cortex or corpus luteum was used, there was poor evidence of function.

In the human transplants it might seem desirable to utilize biopsy material from young donors of proved fertility. In our first case, we chose to use a donor of the same age (15) as the recipient. Ovarian function had been manifest by menarche 3 years previously with irregular and infrequent menses since. The pathological diagnosis of the donor ovarian tissue was "consistent with Stein-Leventhal syndrome." Each of the donors was admitted for exploratory laparotomy for some unrelated complaint. Voluntary permission to donate a

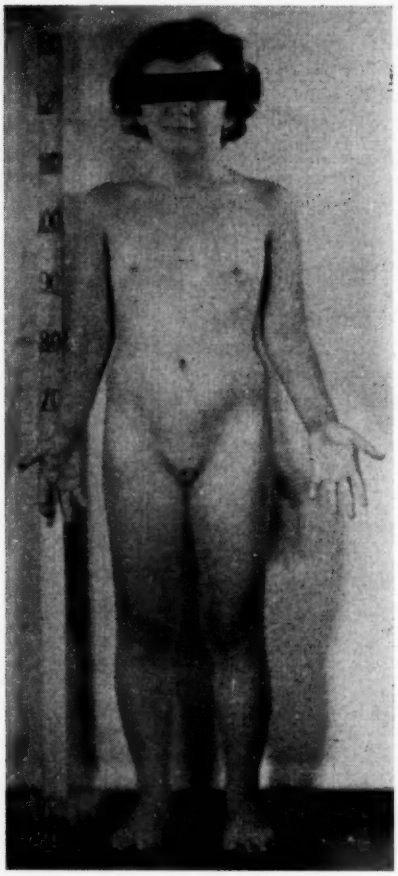

Fig. 9.—Case 5L234.

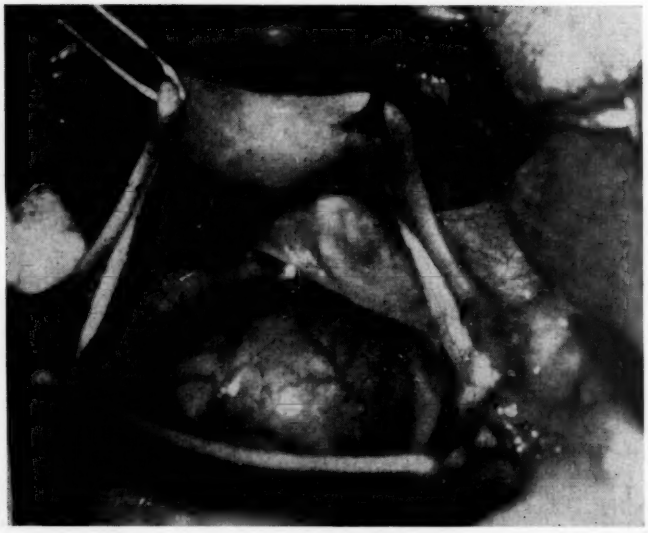

Fig. 10.—Pelvic findings in Case 5L234 showing infantile uterus and lack of development of ovaries.

biopsy of the ovary was obtained each time. The sacrifice of no more than 1 gram of tissue from a normal mature ovary has not been considered detrimental to later ovarian activity.

E. Implantation of Chambers.—In monkey and human recipients a bed is prepared between the leaves of both broad ligaments so that two chambers can be placed as close as possible to the normal site of the ovaries. When Millipore transplants have been placed within the peritoneal cavity, we have not been able to demonstrate function in castrate rats, presumably because any estrogen produced therein is inactivated by the liver before reaching the general circulation. Since it is not possible to crowd more than about 150 milligrams into

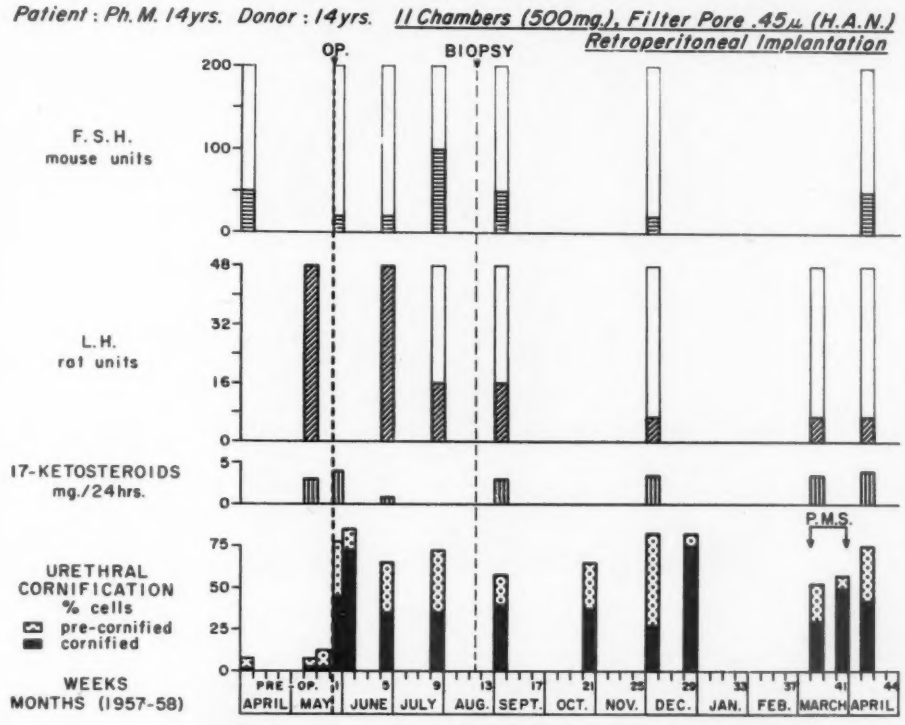

Fig. 11.

each of the 1 inch Millipore wafers and since it has appeared important to use more than this for best functioning, we have had to use other sites for implantation. A paramedian lower abdominal incision is used in order to be able to free readily a space beneath each rectus muscle where four or five more chambers can be embedded, lying on top of the peritoneum. Finally, in each of the human subjects, we have implanted a single chamber subcutaneously at the side of the incision in preparation for its removal under Novocain or Pentothal at a selected time to note the microscopic appearance of the transplanted tissue.

F. Indices of Survival and Function.—

1. In the monkey the vaginal smear provides an easy method of calibrating estrogen production. This is also of course true in the human. We have, however, confirmed the observation by others[7] that the epithelial cytology washed from the proximal urethra in a voided specimen can be used as an index of ovarian function with results comparable to those from the vaginal smear (to be published). It was more practical in the cases here reported—all patients under 16 years of age—to follow the urinary cytology than to insist on repeated vaginal smears before and after operation.

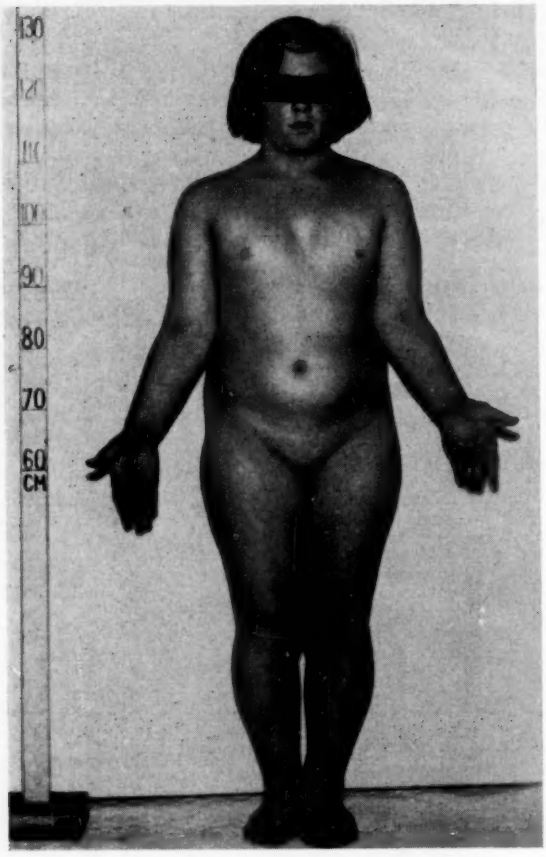

Fig. 12.—Case 5L934.

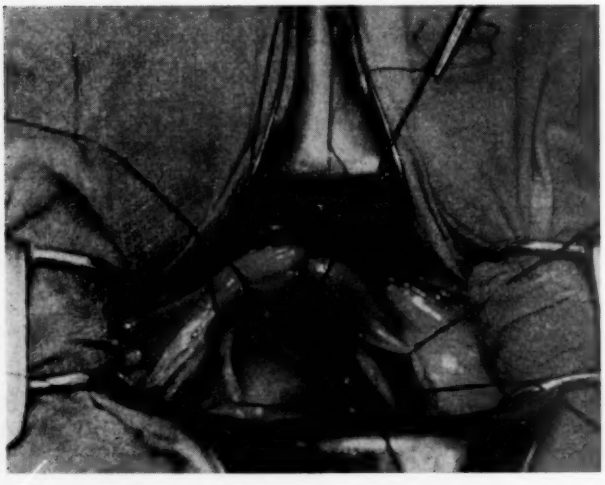

Fig. 13.—Pelvic findings in Case 5L934. The Fallopian tubes are imperfectly fused at the fundus. The ovaries are absent.

2. Levels of pituitary gonadotropins (FSH) were repeatedly tested before and after operation. These were determined on 24 hour urine specimens extracted with kaolin, precipitated, dried, taken up in distilled water, and injected over 3 days in immature mice. An increase in the weight of the uterus of 150 per cent over those of untreated controls was considered a positive test for the dilution of extract used. A positive test for 50 mouse units in 24 hours in a girl with primary amenorrhea of 15 or younger is considered as definite evidence of complete ovarian deficiency, as is a level of 100 or more mouse units in an older woman.

We are unfamiliar with any previous calibrations of gonadotropins in the monkey. Repeated tests of adult female *Macacus rhesus* indicate that the intact animal puts out about 2.5 mouse units or less in a 72 hour sample collected under refrigeration. After castration the level may rise as high as 10 units per 72 hours.

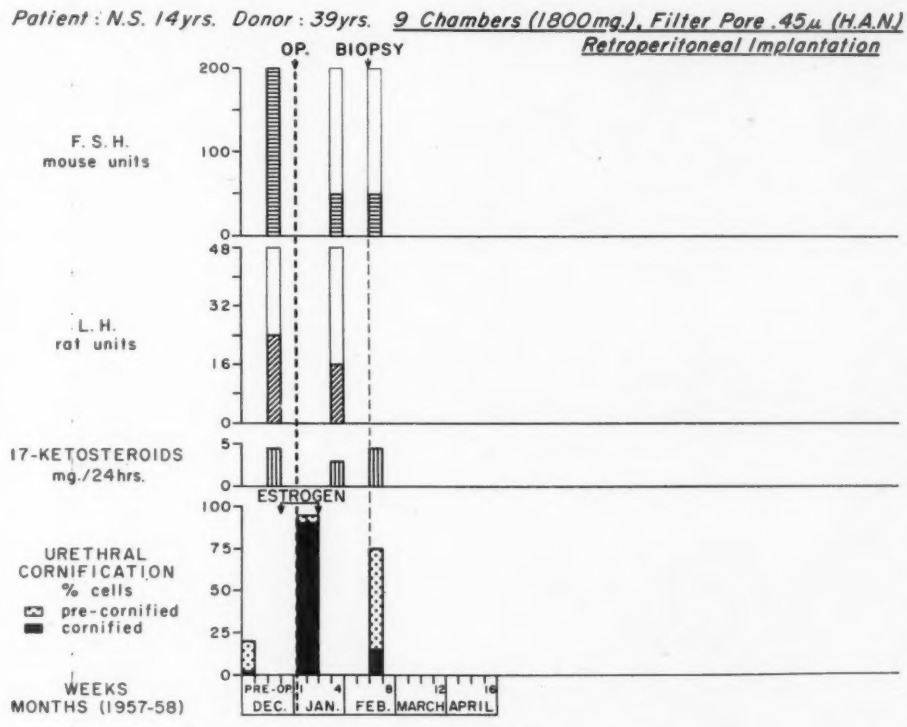

Fig. 14.

The human patients chosen for ovarian transplantation have had further evaluation of gonadotropins by measurements of the increment of ventral prostate weight of immature male rats after injection of kaolin extracts of 24 hour urine samples. This is thought to assay for the LH component. In the normal cycle this varies from a "resting" postmenses level of positive for 8 rat units, to an ovulating but transient rise as high as 48 units as performed in our laboratory.[8]

A persistent reduction in abnormally high preoperative FSH or LH titers after ovarian transplantation can only be considered as evidence of production of ovarian steroid material by the transplanted tissue.

3. Endometrial stimulation has been judged by the occurrence of spontaneous bleeding, by induction of such bleeding with progesterone, and by

endometrial biopsy in the monkey. Although we have given progesterone in the clinical cases here reported, we have not subjected these patients to endometrial biopsy.

Fig. 15.

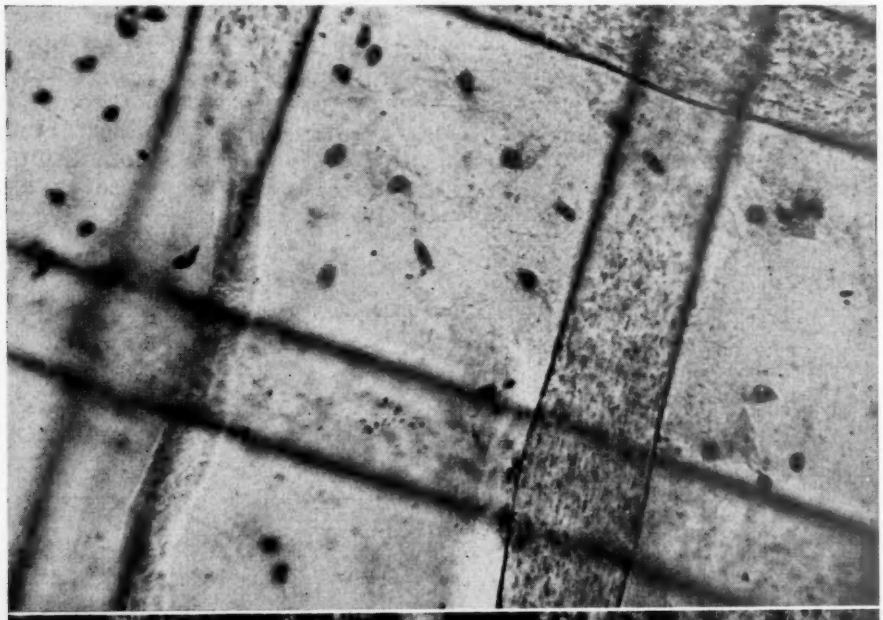

Fig. 16.

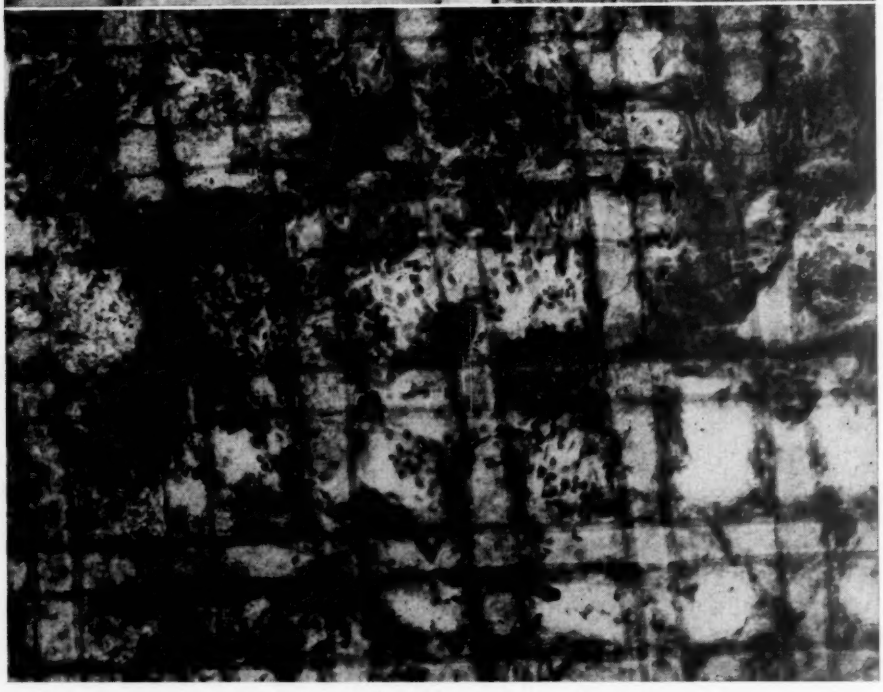

Fig. 15.—Cells from a filter chamber removed 3 weeks after grafting, Case 5L234. The nylon mesh incorporated in the filter appears as a coarse cross-hatching.

Fig. 16.—Cells from a chamber 6 weeks after grafting, Case 5L934.

4. Microscopic review of the transplanted tissue within a chamber removed at varying intervals postoperatively has been available in all our cases. The apparent vigor and integrity of the cells microscopically attests to survival, even though not function, of the transplants.

Fig. 17.

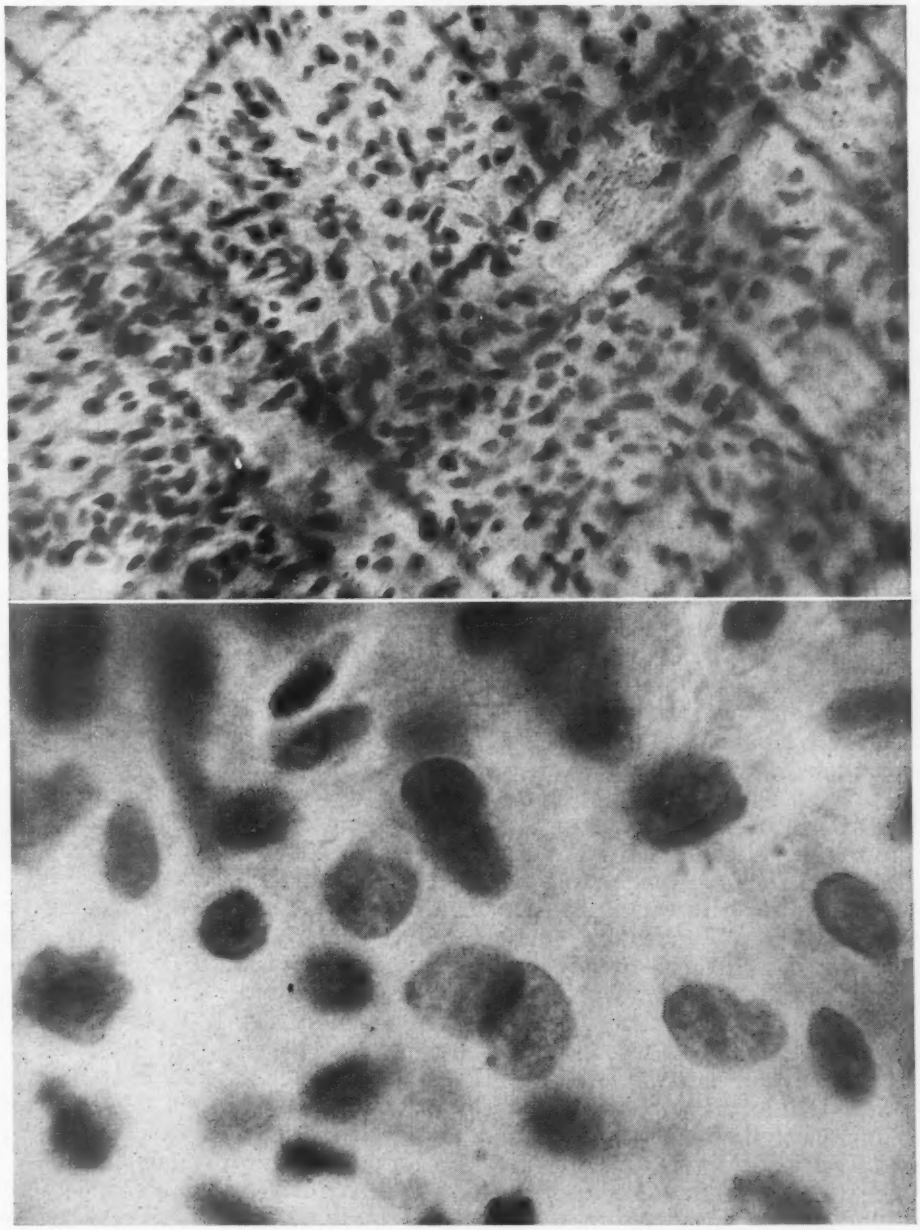

Fig. 18.

Fig. 17.—Cells from a chamber 12 weeks after grafting, Case 9K768. The cells appear to have proliferated to form almost a solid sheet of tissue immediately under the filter membrane. Compare with Fig. 15.

Fig. 18.—High power of Fig. 17.

Results

Preliminary work in castrated female rats showed that implantation of a homotransplant of ovarian fragments in a Millipore chamber transformed an atrophic vaginal smear to one with good cornification. The removal of these nonvascularized grafts was followed by a return to an atrophic smear.[9] We have also reported successful function of similar preparations in monkeys with Millipore chamber ovarian homotransplants and a similar result was found in the heterotransplant of ovary tissue from one type of monkey to another.[6] This prompted us to transplant human ovarian tissue to a castrated monkey. A previously functioning autograft was removed at the time of operation and this probably accounted for a "withdrawal" bleeding episode directly after this operation (Fig. 2). Two and a half months later FSH was still positive for 5 units, indicating low estrogen production, but a month later the vaginal cornification was at a normal level of 75 per cent and bleeding from the endometrium was induced by 10 mg. of progesterone intramuscularly. Five months after transplantation the FSH was negative for 5 units and the chambers were removed. The endometrium at this time shows evidence of estrogen support under low and high power (Figs. 3 and 4), and the tissue removed from within the filter chamber 150 days after grafting looks healthy and viable (Fig. 5).

Based on this experience, we decided to attempt ovarian homotransplants in cases of ovarian dysgenesis. The first patient is seen in Fig. 6. This 14-year-old adolescent, 4 feet, 3 inches tall, showed moderate webbing of the neck, no secondary sexual characteristics, a buccal smear with negative (male) chromatin pattern,[10] and high gonadotropins. A pelvic laparotomy revealed thin white linear conglomerates of fibrous tissue only under two Fallopian structures that united at a rudimentary cervix (Fig. 7). There was no uterus. Her course is seen in Fig. 8. Preoperatively FSH was at 50 M.U. and LH at 48 R.U. levels. The total of precornified and cornified cells in the urine sediment was only 10 per cent. Five hundred milligrams of ovarian tissue was implanted in 11 chambers from a 15-year-old donor with polycystic ovaries, a diagnosis not anticipated before operation. Directly after operation the increase in cornification count and decrease in FSH may be attributed to the amount of estrogen present in the grafted tissue. Seven months later, even though almost full cornification was present and FSH and LH levels were well below preoperative values, there were no changes whatsoever in the patient's clinical appearance. A 3 weeks' course of injections of pregnant mare's serum has induced no clinical changes. The FSH level is once again at a preoperative level of 50 M.U. A chamber was removed 3 months postoperatively.

The next patient was a 15-year-old child with a negative chromatin test, seen in Fig. 9. At laparotomy she showed typical rudimentary linear ovarian vestiges but a well-formed, though infantile, uterus (Fig. 10). Ten chambers with 1,600 milligrams of ovarian tissue were implanted. In Fig. 11 we see high FSH (positive for 200 M.U.) and low cornification (30 per cent) before operation. A chamber was removed for microscopic analysis 3 weeks postoperatively. Four months later a peak of cornification is seen at a time when FSH and LH levels were both lower than preoperative levels. Eight months after operation, however, the cornification is dropping and the gonadotropins rising to previous levels. There has been no clinical improvement noted.

The third patient, also a chromatin-negative 15-year-old child, is seen in Fig. 12. Her pelvic structures were also typical of ovarian dysgenesis (Fig. 13). Ten chambers with 1,800 milligrams of ovarian fragments were implanted. Fig. 14 indicates high preoperative gonadotropins and low cornification. She was given a "priming" course of estrogen* 2 weeks before operation. Her

*Premarin, 7.5 mg. orally per day.

subsequent course suggests persistent function of the transplants according to the laboratory indices here charted. There has been a suggestive increase in breast tissue. A few scattered pubic hairs are now apparent. A biopsy of the tissue in a chamber was obtained 6 weeks after transplantation. The final three figures show the tissues removed at 3 weeks, 6 weeks, and 12 weeks postoperatively (Figs. 15, 16, and 17). It is interesting to note the sparse distribution of cells at 3 weeks (Fig. 15) compared with those seen between the cross-hatching of nylon threads at 12 weeks (Fig. 17). Under high power, the latter are clearly vigorous and healthy (Fig. 18).

Comment

Two years' experience with successful functioning of ovarian autotransplants and homotransplants of ovarian tissue as nonvascularized grafts in rats and monkeys has previously been reported. Laboratory indices and microscopic sections have convincingly demonstrated the production of estrogen from such Millipore-chamber transplants for at least 7 months. In the monkey, evidence of steroid production was sometimes delayed for as long as 2 months after operation. In the 3 human cases here reported, although suggestive evidence of changes in gonadotropin levels and urethral cytology is noted, these changes have not been sustained and any subjective evidence of clinical improvement due to estrogen has been meager or lacking. In the monkey we have utilized about 600 milligrams of material in multiple chambers. In our clinical cases we have used closer to 2 grams. Sequential biopsies in these cases emphasize that there must be massive loss of fragments in the first few weeks. Possibly only a small per cent of the tissue cells survive. At 3 weeks, the biopsy showed a sparse population of cells but at 6 weeks and 3 months we recovered a diffuse pattern suggesting cellular proliferation.

There is an obvious limit to the sites available for imbedding multiple Millipore chambers. Although we calculated that 2 grams should be sufficient, especially if growth and proliferation occurred, perhaps the loss due principally to anoxia in the first few weeks results in inadequate functioning tissue to produce more than a token amount of estrogen as reflected by a significant increase in epithelial cornification and at least a temporary reduction in pituitary gonadotropins. Of possibly greater significance, however, is the fact that, in these 3 cases, the sex chromatin pattern was of the male type. It may be that the target organs—principally the breast, the uterus, and vagina, conditioned genetically toward the male and only secondarily modified in utero toward the female—are incapable of responding readily to estrogenic stimulation. It is notoriously difficult, for instance, to produce more than moderate breast development with exogenous estrogens in many such cases.

We have recently performed a fourth homotransplant in a case of ovarian dysgenesis with a positive, or female, sex chromatin pattern. Favorable response in this case would tend to confirm the above hypothesis. The importance of these observations lies chiefly in the convincing demonstration that endocrine homotransplants may be protected from destruction by implantation in Millipore filter chambers and may function with production of specific steroids over extended periods. As yet we have no knowledge of the length of time

that these filters will maintain their porosity. We believe that these ovarian transplants establish the probability that nonvascularized elements of other ductless glands can be sustained and expected to exhibit their specific function in such chambers in selected clinical cases.

Summary

Application of methods previously used in successful homotransplants in laboratory animals to human cases has demanded a modification in technique. It appears to be important to schedule simultaneous operations on donor and recipient in order to minimize the time lost in transfer of the grafts. A special clamp and cement have been developed to use with nylon-toughened Millipore membranes. Almost 2 grams of minced ovary distributed in approximately 10 chambers of 1 inch diameter is the maximum we have thus far found it practical to transplant. Although there has been clear evidence of the production of estrogenic steroids by laboratory indices for several weeks to months in 3 cases of ovarian dysgenesis, there has been practically no evidence of mammary growth or pubic or axillary hair. All 3 patients were recorded as chromatin-negative (male) genetic types. It is possible that this factor may be important in the failure of target organ response as compared with that produced by similar homotransplants and a heterotransplant (human to *Macacus rhesus*) in adult female castrated monkeys.

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Discussion

DR. CARL T. JAVERT, New York, N. Y.—Drs. Sturgis and Castellanos have shown painstakingly that grafting of ovarian tissue in the Millipore filter is technically possible. These are nonvascular grafts. Would it not be better to employ more vascular grafts? All of us are familiar with the implantation of the ovum which is a highly vascularized type of graft. Implantation occurs in a vascular, muscular uterus supplied by six arteries. The success of these implants is about 90 per cent with and without mutations; otherwise few of us would be here today.

Drs. Sturgis and Castellanos implanted the Millipore filters in the broad ligaments, the rectus muscle, and subcutaneously. They chose these sites to avoid the portal circulation and destruction of the hormone in the liver. Otherwise the spleen would have been a better site as far as the blood supply is concerned.

Another bloody organ is the urinary bladder which, like the uterus, is also supplied by six arteries with corresponding venous circulation that bypasses the liver. The bladder is also muscular. I believe that vascular, muscular organs are purposeful implantation sites, viz., the human ovum implantation. In addition to a good blood supply, the uterine

contractions squeeze the chorionic hormone of the placenta into the circulation. Accordingly, what does Dr. Sturgis think about transplants into the bladder wall to achieve vascularity and contractility?

It will be a long time before these grafts will be on a practical basis but investigations such as these should be encouraged so as to develop proper techniques. Perhaps in time Dr. Sturgis will transplant the entire ovary. We have all heard of the brilliant transplant of a human kidney recently in Boston. I suppose the rectus muscle as used by Dr. Sturgis is the best available site because of vascularity and muscular contractility. Twenty-five years ago, rectus muscle implants of ovarian tissue were done by gynecologists with some success. Yet the practice has been abandoned. Perhaps Dr. Sturgis will revive it with the Millipore-filter technique.

DR. NORMAN F. MILLER, Ann Arbor, Mich.—Is it not possible to obtain the Millipore filter in larger sizes? Also, what if any, is the tissue reaction around the filter noted at the time of biopsy? Has any effort been made to determine the cell identity in the Millipore filter after it is removed, either by histochemical or other means?

DR. STURGIS (Closing).—The portal circulation has been tried and we get no evidence of estrogenic function when we use these filters free in the peritoneal cavity. One thing we want to stress is that since cellular elements cannot get into these filters neither can the donor cells get out of the filter.

In answer to a question from Dr. Miller, the egg cell itself has never survived in these cells in the filter. The cells themselves are certainly not degenerated toward the fibrocystic reaction but what they are I do not know. Dr. Hertig has seen some of them but he is not sure whether they are theca or not. We are now doing other work to try to identify the structures but we are very pleased at their lack of deterioration to fibrocytes.

Dr. Miller also asked about the size of the filter. The size is purely one's choice. The filters come in sheets about the size of regular manuscript paper and can be cut into any size. We have chosen this size arbitrarily.

There is very little or no tissue reaction around these grafts that we can see, but after three months there is a firm fibrotic capsule that tends to form which I suppose is a foreign-body reaction. We have no information on how long we can expect such a structure to survive and function, but we have been encouraged enough to continue with this work.

DEPARTMENT OF CURRENT OPINION

Re-evaluation

One of the recognized leaders in the management of pregnancy in the diabetic, Dr. Ralph Reis here re-evaluates his stand on this subject. His paper is well worth a careful reading by any physician who ever faces this problem.

On two important topics he modifies his previous stand: (1) in the type of insulin to be used in the immediate predelivery period (and hence the duration of the predelivery hospitalization for diabetic adjustment), and (2) the management of the baby in the immediate neonatal period. Clinicians interested in the management of the patient who coincidentally is diabetic and is pregnant should be acquainted with this re-evaluation by Dr. Reis and his co-workers and hence with his contemporary management of these problems.

PREGNANCY IN THE DIABETIC WOMAN

RALPH A. REIS, M.D., EDWIN J. DECOSTA, M.D., AND ALBERT B. GERBIE, M.D., CHICAGO, ILL.

(From the Department of Obstetrics and Gynecology, Passavant Memorial Hospital and Northwestern University Medical School)

THE increasing number of diabetic women who become pregnant and the resulting interest in this clinical problem warrant frequent review and re-evaluation of accepted management. Our understanding of this problem and our ability to classify patients, as well as to foresee possible problems, have been greatly enhanced by the wide acceptance of the working classification of diabetic women as outlined by White.[1]

This classification is now used almost universally and is heartily recommended. It was designed to separate the benign from the malignant forms of

diabetes and to distinguish between patients with and without vascular changes. Ideally, a careful evaluation of the cardiovascular-renal system should be completed before pregnancy is established, but this is not always possible. We believe that the greatest value of this diagnostic program lies in the fact that it will ultimately help to differentiate between the patients with vascular renal disease and those who have simple toxemia of pregnancy. It also has value as a prognostic indicator and serves as a therapeutic tool in the management of such pregnancies.

The most significant change in our management lies in a modification of attitude toward our previously recommended shift from long-acting to regular insulin prior to termination of pregnancy. It has been demonstrated to us[2] that patients whose diabetes is well controlled by one of the repository insulins, notably NPH, can be maintained on the same regime with only slight modification during the perinatal period. This has been accomplished by using half doses of the daily requirements at 12 hour intervals and maintaining a "6 hour type management" with supplemental crystalline insulin when required.

The advantages of such a regime include a shortening of the period of predelivery hospitalization. In addition, the patient's routine management is upset less and the postnatal stabilization of the diabetes becomes more simple. All of these lead to an increased efficiency of diabetes control. Two points must be remembered. This plan is of value only for patients whose insulin requirements are well established. Second, such patients must be watched for the development of any change in insulin requirements during the immediate postpartum period.

We are still of the opinion that our patients feel better and respond better when they are allowed to have a moderate, controlled glycosuria. This permits the feeling of "well-being" which has been previously emphasized, makes for happier and more responsive patients, and avoids hypoglycemia, a problem which we no longer encounter. In addition, the recent emphasis that diabetes should not be too rigidly controlled particularly in early pregnancy since insulin is teratogenic[3] has strengthened our point of view. This raises the question as to a possible relationship between larger amounts of insulin and the high incidence of fetal malformations in some reported series. Our own incidence of fetal malformation continues to vary between 1 and 2 per cent.

Female sex hormone therapy has not been used in our patients and is not being used at the present time. As has been stated,[4] pregnanediol excretion studies continue too inadequate to be reliable, and estrogen excretion determinations remain extremely variable. We continue unwilling, on the basis of present methods, to accept significant deviations of these substances from the normal and believe that female sex hormone therapy has no place in the management of the pregnant diabetic woman. This position is further strengthened by the appearance of recent reports[5-8] showing that there is an apparent definite increase in masculinization of the female fetus by administration of progesterone in large or continuing amounts during pregnancy. In the light of this recent development, it appears proper to issue again a word of caution against the continuing administration of estrogen and progesterone throughout pregnancy.

Although we have preferred prophylactic oral glucose feedings for the newborn, our present attitude is less rigid than heretofore. There remains a difference of opinion concerning such feedings, and it seems that those who advocate starvation for the first 24 to 48 hours achieve practically the same fetal salvage as do those who insist on routine feedings begun immediately after birth. It should be noted, however, that some groups are now feeding "sick" newborn infants of diabetic mothers promptly and continuously with glucose in saline solution.[9] It may well be that our original recommendation[10] still has merit.

The most important principle in the care of the newborn of diabetic mothers is still found in the recommendations previously made that they must be treated as premature regardless of size, that special attention be given to predelivery analgesia and anesthesia, together with intubation, aspiration of the trachea, and postural drainage to prevent respiratory embarrassment. It is equally important to maintain body heat, avoid anoxia, and to continue constant stimulation of these babies during the first 48 hours of life.

In summary, although these specific methods may change or be changed in the future, the basic principles of management remain the same and should be re-emphasized: careful evaluation of the diabetic woman prior to or early in pregnancy; meticulous diabetic and obstetric management throughout pregnancy, labor, and the puerperium; early termination of pregnancy—usually in the thirty-sixth week; and careful continuing pediatric care of the newborn.

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104 South Michigan Avenue
Chicago 3, Illinois

FURTHER STUDIES ON THE METABOLISM OF N^{15}-LABELED URIC ACID IN NORMAL AND TOXEMIC PREGNANT WOMEN*

JOSEPH SEITCHIK, M.D., ANTON SZUTKA, PH.D., AND CARL ALPER, PH.D., PHILADELPHIA, PA.

(From the Departments of Obstetrics and Gynecology and Biological Chemistry, Hahnemann Medical College and Hospital)

IN A previous communication,[1] the metabolism of uric acid in nonpregnant, pregnant, and toxemic women was studied by the determination of the rate of excretion of parenterally administered N^{15}—labeled uric acid. It was concluded that the quantity of uric acid in normal pregnant women, miscible with the injected uric acid (the miscible pool), was greater than that of nonpregnant women. In acute toxemia, the miscible pool was grossly increased. In addition, it was noted that the production (turnover number) of uric acid was excessive in some patients with acute toxemia, i.e., uric acid was overproduced.

Subsequently, we were troubled by two aspects of that study. The daily excretion of uric acid of the toxemic women varied widely from low to average when compared with our group of normal women. In fact, the highest rates of production of uric acid in our toxemic population occurred in the women with high uric acid excretions. We were concerned with the possibility that all of our toxemic patients, hospitalized in one institution, might have been fed a different "low-purine diet" than the normal pregnant women, hospitalized elsewhere. Contamination of the diet with purines would prevent any valid observations of urate metabolism, inasmuch as we could not differentiate the roles of the diet and cellular metabolism in the production of the urinary urate. In addition, evidence obtained by Bien[2] indicated that the protein content of the diet is a significant determinant of the amount of urate produced and excreted. Neither protein nor calorie intakes were controlled in our previous study.

For these reasons, the study was repeated in 4 normal pregnant, 2 preeclamptic, and one eclamptic women. These patients resided on the metabolic ward, and their diets were rigidly controlled in respect to calories (2,500 per day), protein, and purine during the 4 to 8 days prior to and for the 5 days following the administration of the labeled urate. The techniques were identical with those previously reported with the exception of the better dietary control and the more frequent collection of urine samples during the first 24 hours following the injection of the N^{15}-labeled urate.

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TABLE I. THE METABOLISM OF N^{15} URIC ACID

PATIENT	DURATION OF PREGNANCY 11 WEEKS DIAGNOSIS	NITROGEN (GM./DAY) ORAL INTAKE	NITROGEN (GM./DAY) URINE OUTPUT	PLASMA URATE (MG.%)	URINE URIC ACID NITROGEN (MG./DAY)	INTERCEPT ATOMS % EXCESS SLOPE 1	INTERCEPT ATOMS % EXCESS SLOPE 2	MISCIBLE POOL (GM.) SLOPE 1	MISCIBLE POOL (GM.) SLOPE 2	TURNOVER RATE (POOLS/DAY) SLOPE 1	TURNOVER RATE (POOLS/DAY) SLOPE 2	TURNOVER NUMBER (GM./DAY) SLOPE 1	TURNOVER NUMBER (GM./DAY) SLOPE 2
A	Normal pregnant	5.7	5.6	3.3	159	1.26	0.61	0.85	1.85	1.06	0.55	0.91	1.02
B	Normal pregnant	12.4	9.4	4.0	150	1.35	0.95	0.74	1.12	1.05	0.81	0.78	0.91
C	Normal pregnant	19.4	14.2	4.9	164	1.36	0.78	0.72	1.40	1.20	0.61	0.86	0.86
D	Normal pregnant	19.4	17.1	3.5	203	1.41	0.79	0.70	1.37	1.04	0.50	0.73	0.69
F	Pre-eclampsia	5.7	4.8	6.9	81	0.68	0.58	1.62	1.92	0.48	0.38	0.77	0.74
E	Pre-eclampsia	12.4	8.5	8.8	69	0.56	0.38	1.99	3.05	0.48	0.18	0.96	0.54
G	Eclampsia Hydralazine therapy	19.4	15.8	5.1	108	1.08	0.53	0.96	2.12	0.85	0.42	0.82	0.90

Results

The excretion of N^{15}-labeled uric acid, after its intravenous injection, follows a biphasic pattern (Fig. 1). Each phase is a simple exponential function of time. This is true for all normal and toxemic women studied by us and is apparently characteristic of the excretion of injected urate in man.[3]

The miscible pool is calculated from the ordinate intercepts of the curves, and the turnover rate, from the slopes. The absolute values obtained for a single patient vary with the portion of the curve used for extrapolation. It is presumed that the initial slope (slope 1) and its intercept are determined primarily by factors controlling the rate of distribution of urate (the size of

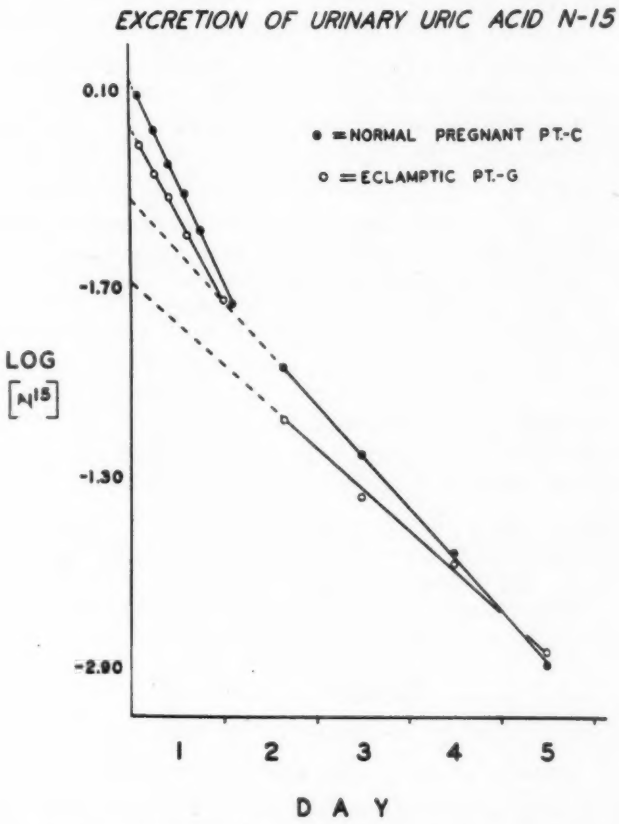

Fig. 1.—The N^{15} concentration in the urinary uric acid is plotted as ordinate, and the time in days, as abscissa. The solid lines approximate measured values. The dotted lines are extrapolations. The miscible pool (*MP*) is calculated from the ordinate intercept (I_0), the quantity of N^{15}-labeled uric acid administered (*UA*), and the concentration of N^{15} in the administered uric acid (I_i): $MP = UA\left(\frac{I_0}{I_i} - 1\right)$. The slopes of the curves are the turnover rates of uric acid (*TR*). The turnover number (*TN*) is the product of the miscible pool and the turnover rate ($TN = MP \times TR$).

the pool, the volume of distribution of urate, capillary permeability, plasma urate binding, etc.). The following slope (slope 2) and its intercept are primarily the net result of metabolic factors (the rates of production, excretion, and metabolic destruction of urate). Therefore, the second slope provides better data concerning the metabolism of urate with less interference from factors concerned with distribution. The data from both slopes are presented in Table I.

In this study, we have the opportunity to observe the effect of toxemia and protein content of the diet on the miscible pool, turnover number, and urinary excretion of uric acid on a few selected patients (Table II):

TABLE II. SUMMARY OF RESULTS

ORAL PROTEIN NITROGEN (GM./DAY)	MISCIBLE POOL (SLOPE 1) (GM.)		TURNOVER NUMBER (SLOPE 2) (GM./DAY)		URINE URIC ACID NITROGEN (MG./DAY)	
	NORMAL	TOXEMIC	NORMAL	TOXEMIC	NORMAL	TOXEMIC
5.7	0.85	1.62	1.02	0.74	159	81
12.4	0.74	1.99	0.91	0.54	150	69
19.4	0.72		0.86		164	
	0.70	0.96	0.69	0.90	203	108

Miscible Pool.—We found the miscible pool to be increased in the 10 toxemic women studied in this and our previous study. The absolute values vary with the ordinate intercepts used for calculation, but the miscible pool is always greater in the toxemic women. The protein content of the diet does not affect the size of the miscible pool in either normal or toxemic women.

Turnover Number.—The pre-eclamptic patients manifest slightly lower turnover numbers than the normal pregnant women when the data is obtained from slope 2. There was some suggestion of an inverse relationship between the protein content of the diet and the turnover number; the lower the protein intake, the higher the turnover number. The eclamptic patient on hydralazine therapy had a lower miscible pool and a higher turnover number than the pre-eclamptic patients. Because of the multiple factors present in the eclamptic patient (eclampsia, treatment, and protein intake), it is difficult to present any interpretation of the results in her case. However, they more closely approximate those of the normal than of the pre-eclamptic patients.

Uric Acid Excretion.—At all levels of protein intake, the uric acid excretion of the toxemic patients was lower than that of the controls. Plasma urate concentration was higher in the toxemic patients, however. In both normal and toxemic patients, the highest level of protein intake (19.4 Gm. per day) produced the highest rate of urinary urate excretion.

Comment

Failure in the previous study to recognize the biphasic nature of the excretion curve of intravenously injected uric acid and failure to control the protein intake resulted in the incorrect conclusion that urate was overproduced in acute toxemia of pregnancy. The limited evidence obtained from this study suggests that urate turnover is somewhat reduced in the 2 pre-eclamptic patients studied.

In the total of 10 toxemic women observed in this and our previous reported work, however, the miscible pool of urate has been increased consistently. Urate is accumulated by toxemic women. There is now no evidence that this is the result of altered metabolism. The elevated plasma concentration and the low renal excretion of urate imply that inadequate renal function is the prime cause of the increased pool of urate in toxemia. Inadequate renal excretion of urate has been demonstrated in toxemic patients by means of precise renal function techniques. The data presented by Chesley,[4] Seitchik,[5] and Hayashi[6]

demonstrate that the excessive tubular reabsorption (or inadequate tubular excretion) of urate is a most consistent defect in toxemic patients. For these reasons we must conclude that the miscible pool of urate is grossly increased in toxemic patients as the result of inadequate renal excretion.

The results of the study were disappointing for it was hoped that the techniques used might provide a measure of the limitation of growth often found in the conceptuses of patients who suffer acute toxemia. The turnover of uric acid, however, measures only the rate of production of urate and, inferentially, the rate of nucleoprotein catabolism. It is possible that the limitation of growth might manifest itself in a decreased rate of nucleoprotein synthesis without any great alteration in the catabolic rate.

Summary

Uric acid labeled with N^{15} was administered to 4 normal pregnant women, 2 pre-eclamptic patients, and one patient with eclampsia during their residence on a metabolic ward under strict dietary control. It was our consistent observation that the uric acid pool was enlarged and the urinary uric acid excretion was reduced in these toxemic women. The rate of production of urate was not increased as was reported previously. The sources of error in the initial report were discussed. It is concluded that faulty renal function is the sole causative factor in urate accumulation in acute toxemia.

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Reviews and Abstracts

EDITED BY LOUIS M. HELLMAN, M.D.

REVIEWS OF NEW BOOKS

The Psychology of Medical Practice. By Marc H. Hollender. 276 pages. Philadelphia, 1958, W. B. Saunders Company. $6.50.

If psychiatry as a dynamic science has found itself isolated for many years from the other medical specialties, the use of its particular jargon was certainly in part responsible. It is also true that only lately has its importance been reluctantly accepted by specialists in many fields. The language used by the author in this work is at all times clear and free of the terminology so often dear to psychiatrists, and frightening to the uninitiated.

The book is divided into chapters dealing with specific medical problems and specialties such as "The Surgical Patient in Health" and "The Pediatric Patient in Illness." It is unfortunate that in some of the chapters, particularly "The Medical Patient" and "The Obstetrical Patient," only specific topics are really dealt with, making these chapters of less value than the others. The introductory chapter on "Patient-Doctor Relationship," the chapter on "The Surgical Patient," and the concluding chapters on "The Psychological Consideration in the Use of Medications," and "The Non Medical Prescription" yield a considerable amount of practical information. The theories from which this information is derived serve to clarify the material instead of confusing the already overburdened mind of the non-psychiatrically oriented specialist.

Some of the recommendations contained in the volume may be questioned, but then the material is not presented in a dogmatic fashion.

The approach adopted by the author of dealing with each topic within the framework of each specialist's needs render this book interesting, valuable, and palatable.

Abortion in the United States. Edited by Mary Steichen Calderone. 224 pages. New York, 1958, Hoeber-Harper. $5.50.

Much of the material discussed at the 1955 conference on illegal and therapeutic abortion sponsored by the Planned Parenthood Federation of America is presented. The book consists of a series of short addresses by many experts in the fields of medicine, biology, sociology, and law, followed by the questions and answers which developed after each address.

It was agreed unanimously that illegal abortion is a major problem in the United States but no one was at all certain of its exact magnitude. According to the late Alfred C. Kinsey, of 5,293 white married women studied, one out of 10 became pregnant either before marriage or after divorce. Of these pregnancies nearly 90 per cent ended in illegal abortion.

By 45 years of age 22 per cent of 5,293 women had had one or more illegal abortions. In this sample 87 per cent of the abortions were reported to have been done by physicians. On the other hand, during the years 1946 through 1953 in New York County only 136 cases of abortion were prosecuted. During this 7 year period there were 411,413 births and about 20 fatal cases of abortion *each year.*

A most interesting part of the meeting must have been the frank report made by a convicted physician abortionist. He had performed abortions on 5,210 women. Of these 35 per cent were single, 53 per cent married, and 12 per cent divorced, widowed, or separated. Only 402 of the subjects had more than 2 children. It was claimed that his patients were referred to him by 353 M.D.'s.

Nearly 10 per cent of the abortions were performed by him on women who were associated in some way with the medical profession. Their number and relationship to the medical profession are tabulated below:

Patient is M.D.	7
Wife of M.D.	58
Relative of M.D.	12
Graduate nurse	270
Student nurse	20
Patient is medical student	4
Wife of medical student	4
Sex partner of M.D. or medical student	26

He stated that another physician abortionist in the same large town had performed nearly a thousand abortions a year for 50 years.

The problems surrounding therapeutic abortion in the United States were discussed at length. The rather impossible legal codes existing in most states today were discussed and compared to the laws regulating therapeutic abortions in the Scandinavian countries. For instance, in the United States, neither rape or incest, even in the very young girl, nor any other humanitarian or social reason is legal ground for abortion.

This conference succeeded most admirably in pointing out the need for extensive revision of the laws covering abortion in the United States. It recommended that the professional organizations in the various fields of medicine, law, religion, sociology, and education should recognize the present importance and ramifications of the abortion problem. Such professional organizations then might be instrumental in developing a body of informed opinion among the citizens of this country, so that solutions to the abortion problem would be approached soberly, and in the most enlightened and democratic manner possible.

This book is most interesting but somewhat difficult to read. The difficulty does not arise from lack of clarity of expression by the members of the conference but from the complex nature of the problem.

Psychoendocrinology. By Max Reiss. 208 pages, 36 figures, 22 tables. New York, 1958, Grune & Stratton. $7.00.

A collection of articles by 23 contributors presented at the Symposium on Psychoendocrinology at the Second International Congress for Psychiatry held at Zurich are presented in "Psychoendocrinology."

Much of the material is highly theoretical and based on small series of cases. It is very apparent that any conclusions drawn are often questionable and the author admits this. Although there are correlations between the disturbances of the endocrine system and mental behavior, and vice versa, there is insufficient knowledge at present to be dogmatic as to any cause and effect.

It appears that the closest relationship between psychiatric disturbances and aberrations of endocrine function occurs in abnormalities of the gonads. In particular, premature or delayed gonadal development may be associated with marked psychic disorders.

The constant theme throughout the book is the careful appraisal of the mentally disturbed patient for possible endocrine imbalances. Correction of these may assist greatly in the treatment of the psychiatric problem.

The need for this book will be very limited and its price will not encourage its purchase.

Die Naturliche Geburt. By Dr. med. Thomas Rust. 105 pages, 73 figures, 42 plates. New York, 1956, Intercontinental Medical Book Corporation. DM 12.80.

A most fascinating subject, viz., natural childbirth, is presented by Dr. Rust. The approach is twofold. The first attack upon the maternal mind is made by lectures so the physician may explain the possibility of going through labor and delivery with some discomfort but with bearable pain. He points out that obviously only a small number of rather intelligent women will qualify for the method. Following this, the women are sent to a school to learn certain exercises to relax certain muscle groups and also to learn how to breathe properly. One gets the impression, however, that most of the exercises keep the patient preoccupied during a painful contraction rather than enhance the labor from a physical standpoint. Ideally, this method would be a panacea for the patient and for the obstetrician if it worked in any large percentage of cases. Unfortunately, its clinical application is limited.

This reviewer's personal experience in the delivery of patients so trained in Switzerland is limited but the results were not impressive. The book itself is well written and interesting. There are numerous illustrations of the performance of the exercises in addition to excellent directions in the text. The book is written in relatively simple German, and is short, but the subject matter leaves something to be desired.

Care of the Premature Infant. By Evelyn C. Lundeen and Ralph H. Kunstadter. 367 pages, 87 illustrations. Philadelphia, 1958, J. B. Lippincott Company. $8.00.

An excellent book, this fills a very definite need by members of the nursing groups. The latest advancements in the care of premature infants are discussed in interesting fashion. With the centralization of premature infants into Premature Centers this book is a "must" as a reference book. The chapter on personnel is very well outlined and invaluable, as is also the one on organization of a Premature Center or Nursery. The illustrations and descriptions of equipment and techniques are excellent.

BOOKS RECEIVED FOR REVIEW

The Author, Publisher, Printer Complex. By Robert S. Gill. Third edition, 134 pages, 8 figures. Baltimore, 1958, Williams & Wilkins Company. $2.25.

Clinical Obstetrics and Gynecology, Vol. 1, No. 2. **Symposium on Toxemias of Pregnancy,** edited by Louis M. Hellman, and **Symposium on Fibromyomas of the Uterus,** edited by Robert A. Kimbrough. 544 pages, 16 tables, 45 figures. New York, 1958, Hoeber-Harper.

Les Déchirures obstétricales compliquées du perinée, traitement chirurgical. By René Musset, Marcel Cottrell, and Maurice Dubost. 120 pages, 19 figures, 22 plates. Paris, 1958, Masson & Cie. 1,400 fr.

Homosexuality, Transvestism and Change of Sex. By Eugene de Savitsch. 120 pages, 7 figures. Springfield, Ill., 1958, Charles C Thomas, Publisher. $3.50.

Human Blood in New York City—A Study of Its Procurement, Distribution and Utilization. By Committee on Public Health, New York Academy of Medicine. 147 pages, 8 figures, 28 tables. New York, 1958, New York Academy of Medicine.

Human Parturition. By Norman F. Miller, T. N. Evans, and R. L. Haas. 248 pages, 67 figures. Baltimore, 1958, Williams & Wilkins Company. $7.50.

Obstetrical Practice. By Alfred C. Beck and Alexander H. Rosenthal. Seventh edition, 1,115 pages, 956 figures. Baltimore, 1958, Williams & Wilkins Company. $14.00.

La Préparation à l'accouchement sans crainte. By F. Lepage and G. Langevin-Droguet. 84 pages, 71 figures. Paris, 1958, Masson & Cie. 900 fr.

Son or Daughter by Choice. By August J. von Borosini. 120 pages, 5 tables, 2 figures. Amsterdam, 1958, E. F. Steinmetz.

A Synopsis of Surgical Anatomy. By Alexander Lee McGregor. Eighth edition, 808 pages, 766 illustrations. Baltimore, 1957, Williams & Wilkins Company. $7.00.

Year Book of Cancer (1957-1958 Year Book Series). By Randolph Lee Clark, Jr., and Russell W. Cumley. 523 pages, 35 tables. Chicago, 1958, The Year Book Publishers, Inc. $8.00.

SELECTED ABSTRACTS

Journal of Clinical Endocrinology and Metabolism

Hawker, R. W.: Oxytocin in Lactating and Nonlactating Women, p. 54.

Blood was collected from lactating and nonlactating women with babies 3 to 7 months old and tested for oxytocin.

The oxytocic activity of the mother's blood collected immediately prior to breast-feeding the infant did not differ significantly from that during suckling or midway between 4 hourly feedings. It did not differ significantly from that in the blood of non-lactating mothers.

The conclusion is drawn that in these studies on human subjects neither oxytocin nor oxytocic substance induces the ejection of milk.

J. EDWARD HALL

Vol. 18, March, 1958.

Sandberg, A. A., and Slaunwhite, W. R., Jr.: The Metabolic Fate of C^{14}-Progesterone in Human Subjects, p. 253.

Before the availability of labeled steroids, little was known about the metabolic fate of progesterone in human subjects.

In this study, C^{14}-progesterone was injected intravenously into 9 human subjects, 4 of whom had bile fistulas. Slightly over 50 per cent of the radioactivity was excreted in the urine. About 40 per cent of the radioactivity was in the form of glucouronidates. Nearly 30 per cent of the radioactivity was excreted in the bile, mostly as conjugates. There was some reabsorbability of the biliary metabolites from the feces and these were not excreted in the urine.

The recovery of total radioactivity in the urines and stools of the non-fistula subjects averaged 67 per cent, in contrast to 82 per cent recovered in the urine, bile, and feces of the subjects with bile fistulas.

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Fajans, S. S.: Hyperthyroidism in a Patient With Postpartum Necrosis of the Pituitary, p. 271.

A case is presented of postpartum necrosis of the pituitary with evidence of panhypopituitarism for 15 years following uterine hemorrhage. Three years after initiation of therapy with cortisone and desiccated thyroid and 18 years post partum, thyrotoxicosis developed. Active hyperthyroidism persisted, but there was continued evidence of pituitary insufficiency with respect to secretion of gonadotrophic, thyrotrophic, and adrenocorticotrophic hormones. The evidence suggests that excessive secretion of pituitary thyrotrophic hormone was not the cause of the hyperthyroidism of Graves' disease in this patient.

J. EDWARD HALL

Vol. 18, April, 1958.

Zander, J., Forbes, T. R., Von Munstermann, A. M., and Neher, R.: Two Naturally Occurring Metabolites of Progesterone, Isolation, Identifications, Biologic Activity and Concentration in Human Tissue, p. 337.

The authors have isolated A^4-3-ketopregnene-20α-OL and A^4-3-ketopregnene-20β-OL from ripe follicles, corpora lutea, placentas, and fat tissue. Both compounds have progestational activity by Hooker-Forbes and Clauberg tests and should be regarded as gestagens.

They are metabolites of progesterone. The relative concentrations of both compounds in the placenta in the second to the tenth month of pregnancy and in the ovary during the menstrual cycle and pregnancy have been quantitatively determined and compared to progesterone levels in these tissues. It appears that progesterone is produced prior to ovulation.

J. EDWARD HALL

Vol. 18, May, 1958.

Little, B., Smith, O. W., Jessiman, A. G., Selenkow, H. A., Van't Hoff, W., Eglin, J. M., and Moore, F. D.: Hypophysectomy During Pregnancy in a Patient With Cancer of the Breast, p. 425.

The presentation describes a patient who was 26 weeks pregnant and had breast carcinoma with metastases. She was treated by a primary hypophysectomy and radiation therapy to the breast. Replacement therapy consisted of 75 mg. of cortisone and 90 mg. of thyroid daily. Premature labor was induced because of hormonal evidence of placental deficiency shown by a rising chorionic gonadotropin excretion. A normal 5 pound infant was delivered.

There was no mammary secretion post partum. Pitressin, which had been required for the control of diabetes insipidus after hypophysectomy, was no longer needed. Twelve days post partum, pneumonitis developed and terminated in death 4 weeks later. Autopsy revealed an organizing pneumonitis of both lungs involving all lobes. The only residual cancer consisted of islets of poorly differentiated cells in an axillary node.

The urinary excretion of chorionic gonadotropin, estrogens, and pregnanediol was not affected by the hypophysectomy. Aldosterone was also not affected. With the discontinuance of cortisone, acute adrenal insufficiency developed. It was evident by all determinations that there was a complete lack of endogenous adrenocortical hormone.

J. EDWARD HALL

Vol. 18, June, 1958.

Wilkins, L., Jones, H. W., Jr., Holman, G. H., and Stempfel, R. S., Jr.: Masculinization of the Female Fetus Associated With Administration of Progestins During Gestation—Non-Adrenal Female Pseudohermaphrodism, p. 559.

The writers report 21 cases of female pseudohermaphrodism not associated with congenital adrenal hyperplasia. These individuals were females born with partial masculinization of the external genitals consisting of an enlarged phallus with or without varying degrees of fusion of the labioscrotal folds. The diagnosis was established by finding female chromatin patterns, low excretion of urinary 17-ketosteroids, and absence of progressive virilization. Exploratory laparotomy showed normal ovaries and a normal female genital tract, although in some cases the vagina and urethra opened into a common urogenital sinus. In 15 of the cases the mother had been treated because of threatened or habitual abortion with oral progestin, 17-ethinyltestosterone. In 2 cases the mother had received intramuscular injections of progesterone and in one case both intramuscular progesterone and oral methyltestosterone had been given. In 3 cases, no steroids were administered during pregnancy. The medication with progestins was usually begun before the tenth week of gestation and in most instances between the fourth and sixth weeks.

It is believed that the female fetus is affected only occasionally when the mother receives these steroids, and that such mothers may have an abnormality of either the metabolism of progestins or their transmission across the placenta.

The condition should be diagnosed correctly at birth and the infant reared as a female. No treatment is required except surgical correction of the abnormalities of the external genitals. Normal female development is certain.

J. EDWARD HALL

Items

American Board of Obstetrics and Gynecology

The Part I Examinations of the American Board of Obstetrics and Gynecology are to be held in various parts of the United States and Canada on Friday, Jan. 16, 1959, at 2:00 P.M.

Candidates notified of their eligibility to participate in Part I must submit their case abstracts within 30 days of notification of eligibility. No candidate may take the written examination unless the case abstracts have been received in the office of the Secretary.

Current Bulletins outlining present requirements may be obtained by writing to the Secretary's office.

ROBERT L. FAULKNER, M.D., SECRETARY
2105 ADELBERT ROAD
CLEVELAND 6, OHIO